PROSTAGLANDINS, MENSTRUATION and MENSTRUAL INDUCTION

Iain Thomas Cameron BSc (Hons) MBChB MRCOG MRACOG

Thesis submitted for the degree of

Doctor of Medicine

University of Edinburgh

1987

CERTIFICATE

The contents of this thesis have not been submitted elsewhere for any other degree, diploma, or professional qualification.

The thesis has been composed by myself, and I have been responsible for patient recruitment, clinical management, and laboratory studies, unless otherwise acknowledged.

Iain T Cameron

July 1987

PROSTAGLANDINS, MENSTRUATION and MENSTRUAL INDUCTION

CONTENTS

PART II - PROSTAGLANDINS and MENSTRUAL INDUCTION

ACKNOWLEDGEMENTS

I am indebted to David Baird for his support and advice during the compilation of this thesis, and to Rodney Kelly for invaluable guidance in the laboratory.

I sincerely thank Rose Leask who performed most of the prostaglandin radio-immunoassays and many of the menstrual blood loss assessments, and Harry Boyle and the staff of the Reproductive Endocrine Laboratories, University of Edinburgh, who measured urinary steroids.

The Research Sisters, Anne Cook, Jenny Gray and Alison Michie provided expert patient care - their help was most appreciated.

John Salmon and Salvador Moncada of the Wellcome Research Laboratories kindly supplied 6oxoPGF$_{1\alpha}$ antiserum for preliminary studies - I thank them not only for this, but also for their enthusiasm and encouragement which initiated my interest in the prostaglandins.

Part I of the thesis was supported by a Birthright Research Grant, and I thank the Royal College of Obstetricians and Gynaecologists for the opportunity of carrying out this work. 16,16 dimethyl-trans-Δ_2-PGE$_1$ methyl ester and RU 486 were donated by May and Baker Ltd., Dagenham, and Roussel Laboratories Ltd., Denham, respectively. I thank them, and in particular thank Sue

Pitts of May and Baker for her understanding in setting up various clinical studies over the last four years.

Illustrations have been compiled by an international team of Tom McFetters and Ted Pinner (Edinburgh) and Chris Hayles (Melbourne) - I am grateful to them all and to Dianne Trengrove who has deciphered my writing with apparent ease!

Finally I acknowledge the consent of the Consultant Staff of the Royal Infirmary, Edinburgh for allowing their patients to be recruited for study, and I thank the women themselves for participating - without their considerable help this work would not have been possible.

ABSTRACT

Menstrual disorders present a considerable clinical challenge, accounting for 10% of referrals to the Gynaecology Out Patient Department. In the majority of cases no organic cause can be found, and there has been increasing evidence that local uterine factors may play an important part in the mechanism controlling menstruation. It has been shown that excessive menstrual bleeding is associated with changes in endometrial prostaglandin production, with the degree of blood loss determined by the relative synthesis of prostaglandins with vasoconstrictory properties on the one hand ($PGF_{2\alpha}$) as opposed to those with vasodilatory properties (PGE_2, PGI_2) on the other. Additionally, the interaction between endometrium and myometrium has been studied, providing evidence that the endometrium may supply precursor endoperoxide for myometrial prostacyclin synthesis.

The first section of this thesis has further investigated the role of the prostaglandins in the pathogenesis of menorrhagia. Of 70 women recruited with a subjective complaint of excessive blood loss, the diagnosis was confirmed objectively in 21 (blood loss > 80 mls per month). These women were randomised to receive treatment with one of the recognised medications for menorrhagia (danazol, mefenamic acid, norethisterone or a progesterone-impregnated coil) and the effect on both the degree of blood loss, and endometrial prostaglandin concentration, was assessed. There was a significant reduction in menstrual blood loss in those women treated with danazol or the progesterone-impregnated coil, and in the latter group there was an associated reduction in endometrial prostaglandin concentrations.

A major clinical application of the prostaglandins has been in the field of menstrual induction, and the second part of the thesis has examined the use of a synthetic prostaglandin analogue to interrupt early pregnancy. The treatment has been as effective as vacuum aspiration of the uterus, but at the expense of unpleasant gastro-intestinal side effects. In an attempt to decrease the incidence of these side effects, a reduced dose of prostaglandin has been given both in a controlled-release system, and in combination with the progesterone receptor antagonist, RU 486. Menstruation has been successfully induced with this combination, but nausea and vomiting continue to be seen in 30% of cases.

Finally, the return to ovulation following menstrual induction has been estimated. The median day of ovulation following prostaglandin administration was 24 days (range 16-32 days), and it is suggested that a prolonged delay to ovulation, resulting in asynchrony between the ovarian and menstrual cycles, would present a significant constraint to the use of the prostaglandins for menstrual induction on a regular basis.

CHAPTER 1

DYSFUNCTIONAL UTERINE BLEEDING: A ROLE FOR THE PROSTAGLANDINS

"But nothing could easily be found that is more remarkable than the monthly flux of women. Contact with it turns new wine sour, crops touched by it become barren, grafts die, seeds in gardens are dried up, the fruit of trees falls off, the bright surface of mirrors in which it is merely reflected is dimmed, the edge of steel and the gleam of ivory are dulled, hives of bees die, even bronze and iron are at once siezed by rust, and a horrible smell fills the air; to taste it drives dogs mad and infects their bites with an incurable poison."

Pliny

Since ancient times menstruation has been very much a taboo subject, poorly understood and enshrouded in mystery. However, over the years much interest has been aroused by this monthly endometrial shedding, partly out of a desire to discover something of its underlying mechanisms, but also as a result of the vast clinical problem that disorders of menstruation can present, for it is estimated that excessive menstrual blood loss may affect up to 20% of women during their reproductive years (Jacobs et al, 1965; Hallberg et al, 1966).

Heavy menstrual bleeding, or menorrhagia, may be the result of organic diseases such as endometrial polyps or fibroids, but in the majority of instances, no such underlying lesion can be found, in which case the diagnosis of dysfunctional uterine bleeding can be made (Novak, 1981). In some circumstances, and especially at the extremes of the reproductive career, this dysfunctional bleeding may be the result of a disturbance of ovarian function (Fraser et al, 1973; Van Look et al, 1977), however, in most women with regular but heavy periods, no impairment of the hypothalamo - pituitary - ovarian axis can be demonstrated (Haynes et al, 1979). In consequence, much attention has focussed on the endometrium itself, for it is possible that local factors may play an important part in the mechanisms controlling menstruation and its disorders.

In the 1914 Hunterian Lecture to the Royal College of Surgeons, Beckwith Whitehouse discussed "some of the older views upon uterine bleeding based principally upon clinical observation, in the light of that more exact knowledge which scientific study and laboratory experiment have placed at our disposal". He described the phenomenon of fibrinolysis of menstrual blood within the uterine cavity, and demonstrated an increase in thrombolysis at the time of menstruation itself. In addition he reported confirmation of Blair Bell's observations on the production of myometrial contractions by the injection of uterine secretions, and proposed that "a portion of the uterine secretion is resorbed and serves as a direct stimulus to uterine contraction, thus diminishing the amount of blood flowing to the endometrium."

Although a haemostatic role for uterine contractility would seem unlikely in the non-pregnant state, the importance of an active endometrial principle in the mechanism of menstruation has received support. In his classical experiments observing the growth and degeneration of endometrial explants in the anterior chamber of the eye of the monkey, Markee (1940) observed that the onset of menstruation was characterised by intense vasoconstriction of the spiral arterioles, with accompanying vasodilatation of the surrounding iridal vessels, and he suggested that these vascular changes could be effected by "some agent elaborated by the degenerating endometrium."

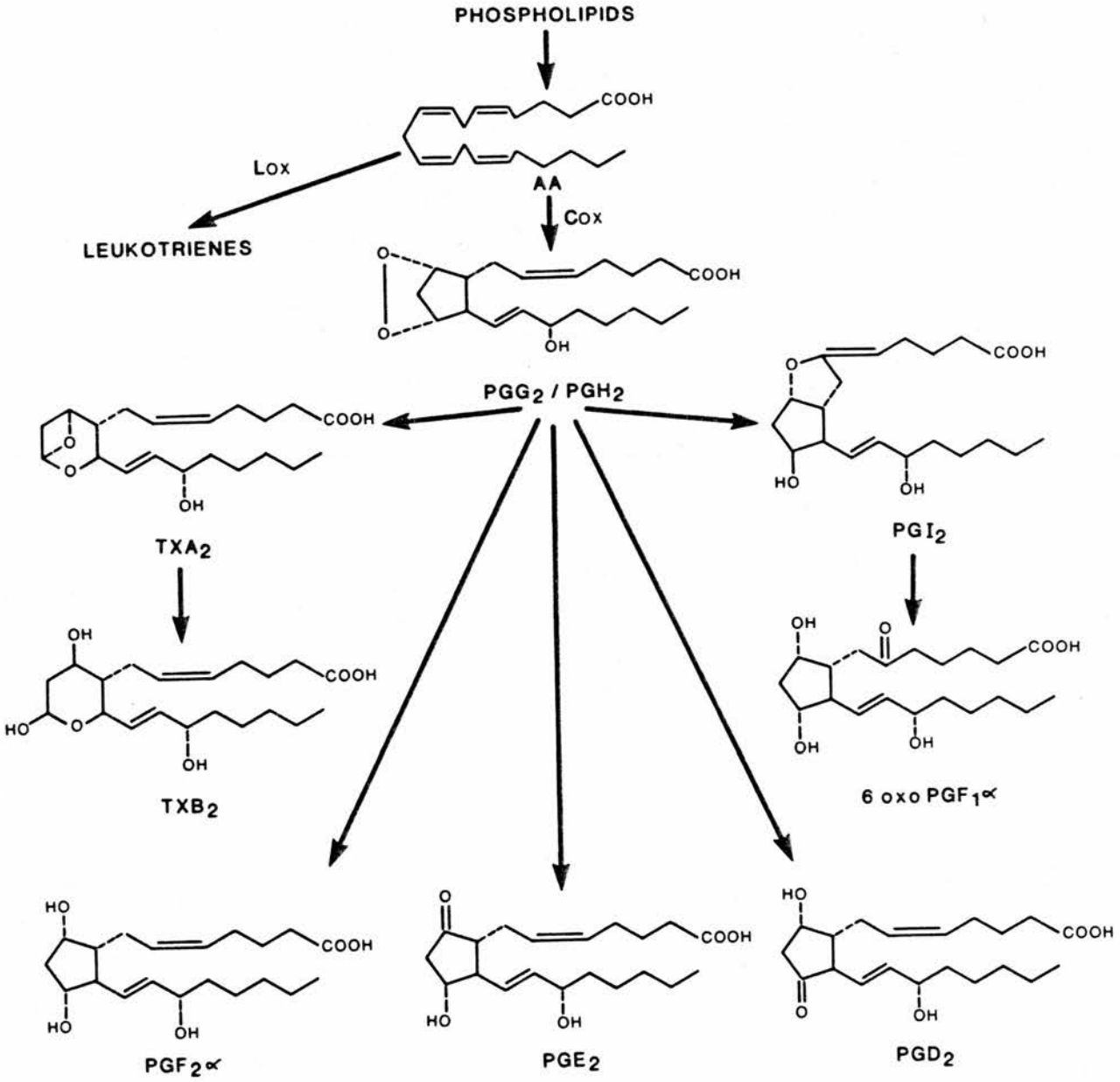
Six years previously, von Euler (1934) had demonstrated a plain muscle stimulating substance from the male accessory genital glands which he had termed "prostaglandin". However it was not until 1957 that Pickles reported a similar finding in menstrual fluid, and the

subsequent confirmation of the presence of the prostaglandins E_2 and $F_{2\alpha}$ in both menstrual fluid and endometrium (Pickles et al, 1965) not only revealed the nature of the menstrual stimulant, but also provided a potential candidate for Markee's vasoactive endometrial agent.

Much data has now accumulated suggesting a role for the prostaglandins (PGs) in the pathogenesis of menorrhagia, but before discussing this further it is appropriate to consider the PGs themselves in more detail.

The Prostaglandins

The principle biosynthetic pathways of the PGs are outlined in figure 1. The PGs themselves are not stored in tissues (Bito, 1975), but are synthesised "on demand" from precursor arachidonic acid. This in turn is released from phosphatidyl - choline in cell membrane phospholipids by phospholipase A_2, or from phosphatidyl-inositol by phospholipase C (Flower, 1980). Thereafter metabolism usually occurs via the cyclooxygenase pathway to produce the cyclic endoperoxides, PGG_2 and PGH_2, or via the lipoxygenase pathway towards leukotriene synthesis. The cyclic endoperoxides are unstable intermediates, and are therefore rapidly converted to either the classical primary PGs (e.g. PGD_2, PGE_2, $PGF_{2\alpha}$), or via thromboxane or prostacyclin synthetase to thromboxane (TX) A_2 and prostacyclin (PGI_2) respectively. The latter 2 compounds have half lives of 40 seconds and 3 minutes (although that of PGI_2 can be extended to 6-12 minutes in blood or autologous cell-free plasma - Orchard and Robinson, 1981), and are converted to their stable products TXB_2 and 6oxo$PGF_{1\alpha}$.


PHOSPHOLIPIDS
COOH
Lox
AA
Cox
LEUKOTRIENES
PGG2 / PGH2
TXA2
PGI2
TXB2
6 oxo PGF1∝
PGF2∝
PGE2
PGD2

The primary PGs are also labile, being almost completely removed from the circulation on a single passage through the lungs, and are metabolised to more stable entities with little pharmacological activity.

The physiological response following activation of the arachidonic acid cascade is dependent on the relative synthesis of these various PGs, which have different effects on different tissues. Pertinent to the reproductive system are their vascular and muscle-stimulating properties. Of the cyclooxygenase products, TXA_2 is a potent vasoconstrictor and stimulant of platelet aggregation, as opposed to PGI_2, which is a vasodilator and the most potent inhibitor of platelet aggregation yet discovered (Gryglewski et al, 1976). Indeed, a specific control mechanism appears to exist between these two PGs in relation to the equilibrium between the platelet and the blood-vessel wall (Moncada & Vane, 1978).

$PGF_{2\alpha}$ has vasoconstrictory properties, and its myometrial - stimulating action has been implicated in the pathogenesis of dysmenorrhoea (Pickles et al, 1965; Chan & Hill, 1978; Lumsden et al, 1983), and in the generation and maintenance of uterine activity in the pregnant uterus (Csapo et al, 1973a; Brennecke et al, 1985). On the other hand, PGE_2 possesses vasodilatory effects (Malik & McGiff, 1976) albeit with one sixth of the potency of prostacyclin.

Prostaglandin inhibitors

The discovery that aspirin-like drugs inhibit PG biosynthesis by binding to the cyclooxygenase enzyme (Ferreira et al, 1971; Smith &

Willis, 1971, Vane, 1971) offered the potential to artificially manipulate the arachidonic acid cascade. Subsequently a number of compounds have been developed which inhibit not only the cyclooxygenase enzyme, but specifically prostacyclin or thromboxane synthesis (Moncada et al, 1976 and 1977; Tyler et al, 1981). Inhibition of the cyclooxygenase enzyme by the non-steroidal anti-inflammatory agents has had the greatest clinical impact, and the use of one such drug, mefenamic acid, will be discussed in detail in Chapters 2 and 3. However, it should be noted that these compounds will not only result in an indiscriminate inhibition of endoperoxide and other PG synthesis, but that they may also direct arachidonic acid metabolism towards the lipoxygenase system (Pakrasi & Dey, 1985).

Uterine prostaglandins

The capacity of the female reproductive tract to synthesise PGs is considerable, and these compounds appear to play a part not only in normal physiological processes, such as menstruation, implantation and labour, but also in various pathological states.

The major endometrial prostanoids are PGE_2 and $PGF_{2\alpha}$ (Pickles et al, 1965) and their production varies throughout the menstrual cycle. PG concentrations are low in the proliferative phase, and increase markedly in the second half of the cycle, but there is debate as to whether the maximum concentrations occur in the mid-secretory phase (Maathius & Kelly, 1978), or at the onset of, and during, menstruation (Downie et al, 1974). The endometrium also synthesises smaller amounts of PGD_2 (Smith et al, 1981a) and 6oxo$PGF_{1\alpha}$ (Rees et al, 1984b; Kelly et al, 1984), though the powerful pharmacological properties of prostacyclin should be borne

in mind when considering its possible physiological role in such low concentrations.

Endometrial thromboxane production appears to be low (unpublished observations cited in Rees et al, 1984b).

The main product of arachidonic acid metabolism in the myometrium is 6oxoPGF$_{1\alpha}$ (Fenwick et al, 1977; Jones et al, 1977; Abel & Kelly, 1979). Although the production rate per gramme of tissue is similar to that in the endometrium itself, total uterine 6oxoPGF$_{1\alpha}$ synthesis is augmented by the large muscle mass of the myometrium. Furthermore, there may be an interaction between endometrium and myometrium, with the former tissue providing precursor endoperoxide for myometrial PGI_2 synthesis (Abel & Kelly, 1979; Smith et al, 1981b).

In addition to the cyclooxygenase products of the arachidonic acid cascade, recent evidence has suggested the presence of lipoxygenase pathways in the uterus (Hahn et al, 1985), with a preponderance of lipoxygenase activity in the myometrium (Demers et al, 1984). However Rees et al (1986) have demonstrated a significantly greater release of the leukotrienes C_4, D_4 and E_4 from endometrium than from myometrium, and have proposed a cyclical production of these compounds with a peak in the mid luteal phase. Major methodological differences will contribute to these conflicting results - in the former study the monohydroxy acids 5-hydroxyeicosatetraenoic acid (5-HETE) and 12-HETE were identified by high pressure liquid chromotography after incubation of arachidonic acid with human platelets, whereas the latter study utilised specific leukotriene antibodies for radioimmunoassay.

Prostaglandins in pathological states: prostaglandins and menorrhagia

Since Pickles' earlier observations (Pickles 1957; Pickles et al, 1965), increased concentrations of $PGF_{2\alpha}$ and PGE_2 have been further demonstrated in the menstrual fluid of women with dysmenorrhoea (Lumsden et al, 1983; Rees et al, 1984a). The PGs have also been implicated in the pathogenesis of menorrhagia. Willman et al (1976) reported an increase in endometrial PGE_2 and $PGF_{2\alpha}$ in both the follicular and luteal phases of the menstrual cycle in women with dysfunctional uterine bleeding, however the degree of blood loss was not measured (see Chapter 2). More recently, using the alkaline haematin method to assess menstrual loss (Hallberg and Nilsson, 1964), Smith et al (1981a and 1982) have defined abnormalities in endometrial PG synthesis in women with both ovulatory and anovulatory menorrhagia. In those patients experiencing regular ovulatory cycles, there was an association between menstrual blood loss (MBL) and the ratio of PGE : $PGF_{2\alpha}$, and, in addition, women with a blood loss >90mls had a significantly greater endometrial concentration of PGE than those individuals with normal menses. It was therefore suggested that excessive bleeding resulted from a shift in endometrial conversion of endoperoxide from the vasoconstrictory $PGF_{2\alpha}$ to the vasodilator PGE_2. In persistent proliferative endometrium however, the abnormality appeared to be an impaired PG synthetic capacity due to a reduced availability of precursor arachidonic acid. Again the ratio of the concentrations of $PGF_{2\alpha}$: PGE was inversely related to the degree of MBL, but the concentration of $PGF_{2\alpha}$ in persistent proliferative and normal secretory

endometrium was also signficantly correlated with the degree of bleeding.

This relationship between $PGF_{2\alpha}$ and measured MBL, though apparently inconsistent with the concept of an imbalance between the vasodilatory and vasoconstrictory PGs, has also been reported in menstrual fluid on the first day of menses, with an associated increase in PGE_2 on the second day (Rees et al, 1984a). However, using a superfusion technique, alternative data from the same laboratory (Rees et al, 1984b) failed to show any correlation at any stage of the menstrual cycle between MBL and the endometrial release of $PGF_{2\alpha}$, PGE_2 or 6oxo$PGF_{1\alpha}$. Both the difficulties of measuring tissue PGs and different techniques of measurement will contribute to such variance in reported data, and this problem will be discussed in Chapter 3.

Ovarian Control

The influence of the ovarian steroids on the control of uterine PGs should also be addressed. The cyclical nature of endometrial PG production has already been mentioned, and it has been suggested that the increased PG production in the luteal phase may be a direct result of the action of progesterone. An apparent paradox exists though, for progesterone itself appears to inhibit both PG release in tissue culture (Abel and Baird, 1980; Tsang and Ooi, 1982; Gurpide et al, 1986), and also PG appearance in the utero-ovarian vein of the guinea-pig (Blatchley and Poyser, 1974). A period of progesterone priming though, followed by estradiol stimulation appears to constitute the optimum conditions for $PGF_{2\alpha}$ synthesis (Abel and Baird, 1980), however this view has

recently been challenged by Schatz et al (1985), who have suggested that the removal of an inhibitory influence during the isolation and culture of endometrial glands may account for the differences in cited results. In summary, it may be that progesterone has a role in facilitating precursor arachidonic acid storage in the luteal phase, resulting in a given potential for PG synthesis, which will be unmasked as steroid concentrations fall with impending menstruation. Such a role could be mediated via an impairment of phospholipase activity (Wilson et al, 1986).

The observed physiological effects of the PGs are related not only to the rate of tissue synthesis, but also to the balance between synthesis and degradation. Although estradiol stimulates cyclooxygenase activity (Ham, 1975), no specific effect is seen on $PGF_{2\alpha}$ metabolism in vitro (Schatz et al, 1985). Progesterone on the other hand stimulates PG metabolism, which is greatest in the second half of the cycle (Casey et al, 1980; Abel and Kelly, 1983) and which can be reduced in vitro using the progesterone receptor antagonist RU 486 (Kelly et al, 1986b). Furthermore, these steroidal effects are inter-related, as estradiol induces progesterone receptors in the human uterus (Janne et al, 1975), whereas progesterone itself suppresses the estradiol receptor (West & Brenner, 1985).

The first part of this thesis aims to further investigate the role of the PGs in the pathogenesis of menorrhagia. Chapter 2 outlines the definition of a group of women with objectively diagnosed ovulatory dysfunctional bleeding, and demonstrates the clinical

effects of four medical treatments (danazol, mefenamic acid, norethisterone, and a progesterone - impregnated coil) on the degree of blood loss. In addition, endometrial biopsies have been obtained to evaluate the relationship between endometrial PGs and MBL, and to assess whether the four medical treatments are associated with changes in the pattern of endometrial PG production. These data are presented in Chapter 3. Finally, Chapter 4 investigates the interaction between endometrium and myometrium in vitro.

CHAPTER 2 (i)

THE OBJECTIVE ASSESSMENT OF MENSTRUAL BLOOD LOSS

The assessment of menstrual blood loss must be based on objective measurement. A subjective account of the degree of blood loss, either in terms of the number of days bleeding or the amount of sanitary protection required is inadequate to diagnose menorrhagia, for it has been shown that up to 50% of women complaining of heavy periods have a measured blood loss within normal limits (Fraser et al, 1981; Dockeray et al, 1986).

Many methods have been used to assess MBL objectively including the weighing of sanitary towels (Pendergrass et al, 1984), and the measurement of haem iron by absorption spectrophotometry (Cole et al, 1971). However the most popular method has been to determine the concentration of haemoglobin in the menstrual effluent by converting it to an alkaline haematin derivative which can be quantified colorometrically (Hallberg & Nilsson, 1964; Newton et al, 1977).

Using the alkaline haematin method, the mean monthly blood loss in one large population study (n=476) was 43 mls (Hallberg et al, 1966). The 95th centile for MBL was 76.4 mls, and 67% of those women with a blood loss in excess of 80 mls showed evidence of iron deficiency anaemia. The development of anaemia will depend to a major extent on the intake of iron, and it has been suggested that if the monthly MBL exceeds 50-60mls, negative iron balance will result on an "average" western diet (Rybo, 1966; Smith, 1982). The upper limit of normal for MBL has therefore been defined as 60-80 mls.

The present study has examined a group of women presenting with a subjective complaint of menorrhagia. MBL has been measured using a

modification of the alkaline haematin method, and the effects of medical treatment on the degree of blood loss have been assessed in those women in whom the diagnosis of heavy menses has been confirmed.

PATIENTS AND METHODS

70 parous women with a subjective complaint of heavy periods were recruited from the Gynaecological Out Patient Department of the Royal Infirmary, Edinburgh. All suffered from dysfunctional uterine bleeding and had undergone diagnostic curettage to exclude underlying organic pathology. None of the women were currently receiving medical treatment for their menorrhagia.

Patients were instructed to collect their sanitary towels/tampons for 2 cycles in order to assess their blood loss objectively. Each woman kept a calendar of her menstrual loss, and in addition 26 patients collected early morning urine samples 2 or 3 times weekly to monitor ovarian function. All patients were specifically asked to avoid self medication with proprietary preparations, particularly those containing aspirin or similar agents.

The women were reviewed in the mid luteal phase of the second study cycle, when those individuals with a mean monthly MBL of <50mls were excluded from further study (see discussion). Those with a greater blood loss received treatment with either danazol, mefenamic acid, norethisterone or a progesterone - impregnated coil, and they were asked to collect their sanitary protection for a further 2 cycles (see Chapter 2(ii)).

Blood was taken for haemoglobin concentration, white cell,platelet, and reticulocyte count, along with estradiol and progesterone concentrations, both before the study, and at each follow up appointment.

Assessment of Menstrual Blood Loss

MBL was assessed objectively using a modification of the alkaline haematin method (Hallberg & Nilsson, 1964). Soiled pads and tampons were placed in molar sodium hydroxide and thoroughly mixed. 24 hours later, an aliquot was taken, and after filtration, its optical density was measured at 450nm. MBL was then calculated by comparing this with the optical density of a peripheral blood sample similarly processed. The diagnosis of menorrhagia was made if the blood loss exceeded 80 mls per month.

Prior to the study the reliability of the technique had been tested using sanitary towels to which known amounts of blood had been added.

Urinary assays

The urinary excretion of total estrogen was measured fluorometrically (Brown et al, 1968), and pregnanediol was estimated using gas liquid chromatography (Chamberlain & Contractor, 1968). Both the total estrogen and the pregnanediol were then expressed in relation to urinary creatinine (estrogen: µgm/gm creatinine, pregnanediol: mg/gm creatinine).

RESULTS

Of the 70 women, 2 became menopausal during the study and a further 3 failed to collect their towels adequately. The results of the remaining 65 are presented.

The median age was 39 years (range 25-50) and the median height and weight 159cm (range 146-169) and 64kg (range 49-89) respectively.

Menstrual Blood Loss

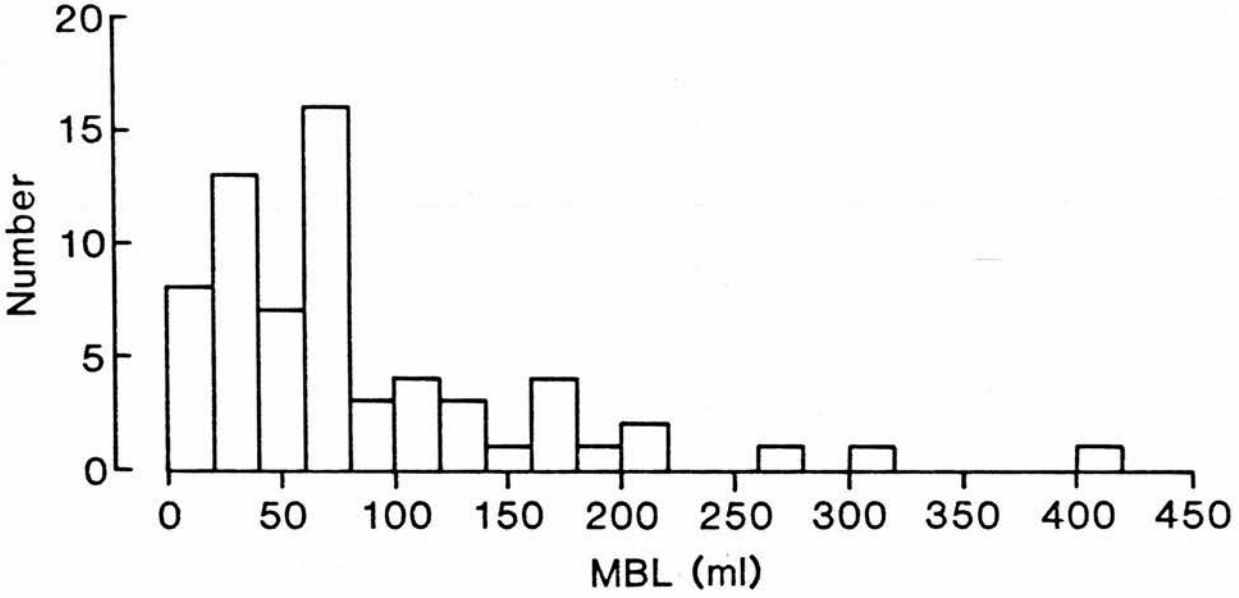
The reliability of the alkaline haematin technique is illustrated in table 2(i)1.

ADDED (mls)	RECOVERED (mls)
5	5.3 ± 0.4
10	10.0 ± 0.3
20	20.8 ± 0.6
40	40.6 ± 0.6
60	61.8 ± 1.5

Table 2(i)1. Validation of the alkaline haematin method. For each volume of blood added, the amount recovered represents the mean ± standard error of 6 experiments.

Figure 2(i)1 demonstrates the range of monthly MBL. The median loss was 63 mls (range 7-429). In 44 (68%) cases the MBL was <80 mls, and it was <50 mls in 27 (42%).

There was no difference between those women with and without objectively diagnosed menorrhagia either in terms of their age, height or weight, or in relation to the duration of menses and cycle length (table 2(i) 2). Although the median parity of the two groups was the same (<80 mls - 2 (range 1-4); >80 mls - 2 (range 1-6)), there were only 2 women who had delivered more than 4 children (parity 5+0 and 6+0 respectively), both of whom were in the heavy blood loss group.


20
15
10
5
0
Number
0
50
100
150
200
250
300
350
400
450
MBL (ml)

	<80mls (39)	>80mls (19)
MBL (mls)	39 (10,78)	156 (86,429)
AGE (years)	38 (25,48)	42 (33,50)
DURATION (days)	5 (2,8)	6 (3,9)
CYCLE LENGTH (days)	28 (23,48)	28 (23,38)

Table 2 (i) 2 Menses duration and cycle length in women with and without objectivily diagnosed menorrhagia. Data are presented as the median with the range in paretheses. The results of 7 women with anovulatory cycles have been excluded.

Endocrine assessment

Serum progesterone was measured in the latter half of the 2nd study cycle. 7 (11%) women exhibited anovulatory cycles (mid luteal progesterone concentration <18 nmol/L), and their characteristics are shown in Table 2(i) 3.

AGE (years)	42 (35,49)
MBL (mls)	31 (7,210)
DURATION (days)	6 (4,9)
CYCLE LENGTH (days)	29 (21,66)

Table 2(i) 3 The menstrual characteristics of 7 women with anovulatory cycles. The data are presented as the median with the range in brackets.

The data from 24 women who collected urine samples to monitor ovarian function are shown in figure 2(i)2. 2 individuals who failed to demonstrate a luteal phase rise in pregnanediol excretion have been excluded from analysis. There was no difference between those women with or without objectively diagnosed menorrhagia in terms of the urinary excretion of both total estrogen and pregnanediol.

Haematological indices

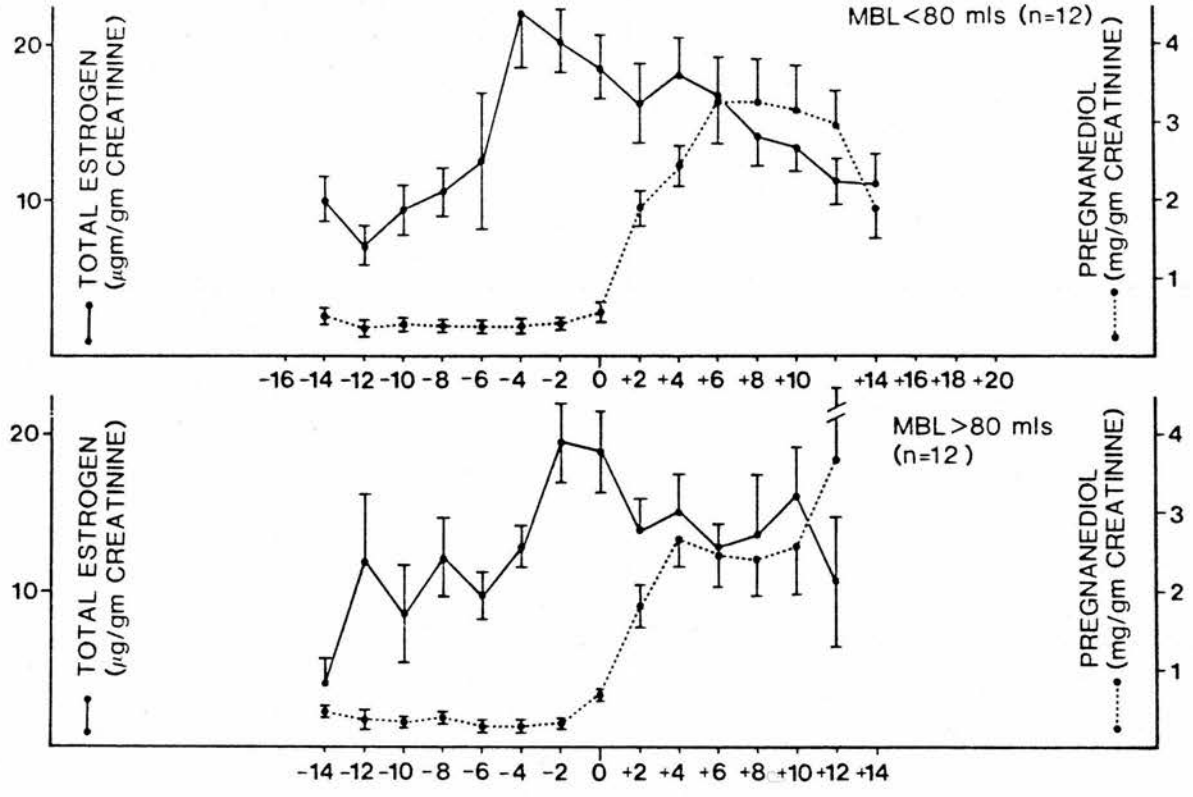
Haemoglobin concentration, white cell, platelet, and reticulocyte count did not differ between those women with or without heavy periods. Table 2(i) 4 shows the median haemoglobin concentration and platelet count for those women with a blood loss greater than or less than 80 mls, and for those women with anovulatory cycles.

	MBL < 80mls(39)	MBL > 80mls(19)	ANOVULATORY(7)
Hb (g/dL)	13.3 (9.5,14.8)	12.6 (10.0,15.0)	13.6 (11.0,14.3)
Platelets ($x10^6$/ml)	228 (126, 330)	234 (160,446)	224 (175,275)

Table 2(i)4 Haematological indices. Data represent the median, with the range in brackets (Hb = haemoglobin).

DISCUSSION

In 1904 Hoppe-Seyler et al. used an acid haematin method to measure MBL in 15 women, 11 of whom were considered to have pathological


MBL<80 mls (n=12)
TOTAL ESTROGEN (μgm/gm CREATININE)
PREGNANEDIOL (mg/gm CREATININE)
20
10
4
3
2
1
-16 -14 -12 -10 -8 -6 -4 -2 0 +2 +4 +6 +8 +10 +14 +16 +18 +20
MBL>80 mls (n=12)
TOTAL ESTROGEN (μg/gm CREATININE)
PREGNANEDIOL (mg/gm CREATININE)
20
10
4
3
2
1
-14 -12 -10 -8 -6 -4 -2 0 +2 +4 +6 +8 +10 +12 +14

gynaecological disease. In the 4 "normal" cases, the blood loss ranged from 26-52 mls with an average of 37 mls. Since this time, many techniques have been employed in an attempt to quantify both normal and abnormal MBL. Measuring menstrual blood iron in 100 apparently normal women, Barer and Fowler (1936) found an average loss of 51 mls (range 7-179), and in 50% of women the loss was between 23 and 68 mls. In addition they noted that the duration of the period, or the number of "napkins" used, gave only a vague indication of the actual degree of blood loss itself.

Other methods have been to weigh sanitary towels - which provides an estimate of <u>total</u> menstrual loss (blood and fluid) if collected adequately (Pendergrass et al, 1984; Fraser et al, 1985) - or to measure menstrual iron using absorption spectrophotometry (Cole et al, 1971). The latter study assessed blood loss in 348 women in a Northumberland mining village: the median blood loss of 28 mls (range 0.1-280) was similar to that of 30 mls cited by Hallberg et al (1966) in Gottenberg using the alkaline haematin approach (Hallberg and Nilsson, 1964).

The alkaline haematin method has become the most widely used technique for MBL assessment (Haynes et al, 1977; Guillebaud et al, 1978). The procedure is accurate, as shown in the present study, provided the patient is instructed to collect <u>all</u> her menstrual loss. However it only measures the haemoglobin component of the menstruum, and although it is this constituent that is relevant in relation to the development of anaemia, recent studies have suggested that in normal women the haem element only comprises 36% of the total menstrual loss (Fraser et al, 1985). This may partly

explain the discrepancy between measured and perceived loss in the present and many previous studies (Fraser et al, 1984).

Here, the finding that 68% of the women with a complaint of menorrhagia had a blood loss <80 mls is in accord with other data (Fraser et al, 1981; Dockeray et al, 1986). This again reinforces the necessity for objective assessment in the diagnosis of heavy blood loss.

As with the Swedish work (Hallberg & Nilsson, 1966) a blood loss of >80 mls has been required to diagnose menorrhagia. Nevertheless it was considered appropriate to treat women with a blood loss >50 mls. Firstly, even with strict patient cooperation, the MBL is likely to be an underestimate. Secondly, the risk of developing anaemia will gradually increase as the blood loss extends towards the upper limit of normal, and women should ideally receive treatment prior to developing frank iron deficiency. Finally, in 21 asymptomatic women presenting for laparoscopic sterilisation in Edinburgh, no patient had a blood loss >50 mls (Smith, 1982).

89% of the women in the present study showed a luteal phase increase in serum progesterone concentration compatible with ovulation, and of the 7 individuals who failed to ovulate, only 2 had a blood loss >80 mls (156 and 210 mls respectively). The similarity between the endocrine profiles of those women with and without menorrhagia was seen in figure 2(i) 2, and these data confirm the results of others (Haynes et al, 1979).

Although menorrhagia is the most common cause of iron deficiency anaemia (Cohen & Gibor, 1980), in the present work there was no

difference in the haemoglobin concentration of those women with heavy periods, compared with those whose blood loss was in the normal range. Furthermore, there was no compensatory increase in the reticulocyte count of those women in the heavy blood loss group. However, the women recruited for study form a biased group, for those individuals with excessive menses resulting in gross iron deficiency anaemia are more likely to be treated surgically rather than to be referred back to the Out Patient Clinic for medical therapy after their diagnostic curettage. In addition, despite the normal haemoglobin concentration, there may be differences in the iron stores between the 2 groups with normal and heavy menses, though these parameters have not been measured here.

In conclusion, these data confirm the importance of objective assessment in the interpretation of menstrual abnormalities. The next section of this Chapter examines the efficacy of medical treatment in those women with a measured blood loss >50 mls.

CHAPTER 2(ii)

THE OBJECTIVE ASSESSMENT OF MEDICAL TREATMENT FOR MENORRHAGIA

Numerous methods are available for the treatment of heavy MBL ranging from the combined contraceptive pill (Nilsson & Rybo, 1971) or norethisterone (Bishop & Almeida, 1960), to the use of prostaglandin (Anderson et al, 1976) and fibrinolytic inhibitors (Ylikorkala & Viinikka, 1983) or the insertion of medicated intra-uterine devices (Cohen & Gibor, 1983). Although such treatments are used widely for the management of menstrual disorders, in many instances neither the original diagnosis, nor the subsequent response to therapy, have been evaluated objectively.

The profile of MBL in a group of 65 women complaining of heavy MBL was shown in figure 2(i)1, where 38 (58%) individuals had a blood loss in excess of 50 mls. The present study has assessed the effects of medical treatment on 30 of these women.

PATIENTS AND METHODS

30 women with a mean monthly blood loss of > 50 mls were recruited for further study from the original group described in Chapter 2(i). All had ovulatory menstrual cycles.

They were randomly allocated to receive treatment with danazol (200mg daily), mefenamic acid (500 mg three times daily during menses), norethisterone (5mg twice daily from day 15-25 of cycle) or a progesterone-impregnated intrauterine device (releasing 65 µgm progesterone daily). Patients were instructed to collect their soiled sanitary towels for a further two (mefenamic acid) or 3 cycles (danazol, norethisterone and progesterone coil), and were

reviewed in the mid luteal phase preceding their last study period. At this stage blood was taken for haemoglobin concentration, white cell, platelet and reticulocyte count, and estradiol and progesterone concentrations. In addition some women underwent endometrial biopsy to assess PG concentrations, and these data will be presented in Chapter 3.

Assessment of Menstrual Blood Loss

All patients kept a calendar to chart the duration of each period, and the interval between periods. MBL was objectively assessed using the alkaline haematin method as previously described.

RESULTS

The characteristics of the 30 women are shown in table 2(ii)1. There were no significant differences between the 4 treatment groups.

TREATMENT(n)	AGE(yrs)	HEIGHT(cm)	WEIGHT(kg)	PARITY
DANAZOL (6)	42(36,50)	157(149,169)	64(52,76)	2(1,6)
MEFENAMIC ACID(8)	40(33,48)	162(149,164)	64(50,70)	4(2,4)
NORETHISTERONE(8)	39(35,46)	164(162,169)	64(52,73)	4(1,4)
PROGESTERONE COIL(8)	40(29,42)	162(145,164)	70(54,89)	2(2,4)

Table 2(ii)1 Patient characteristics. The median value is expressed with the range in parentheses.

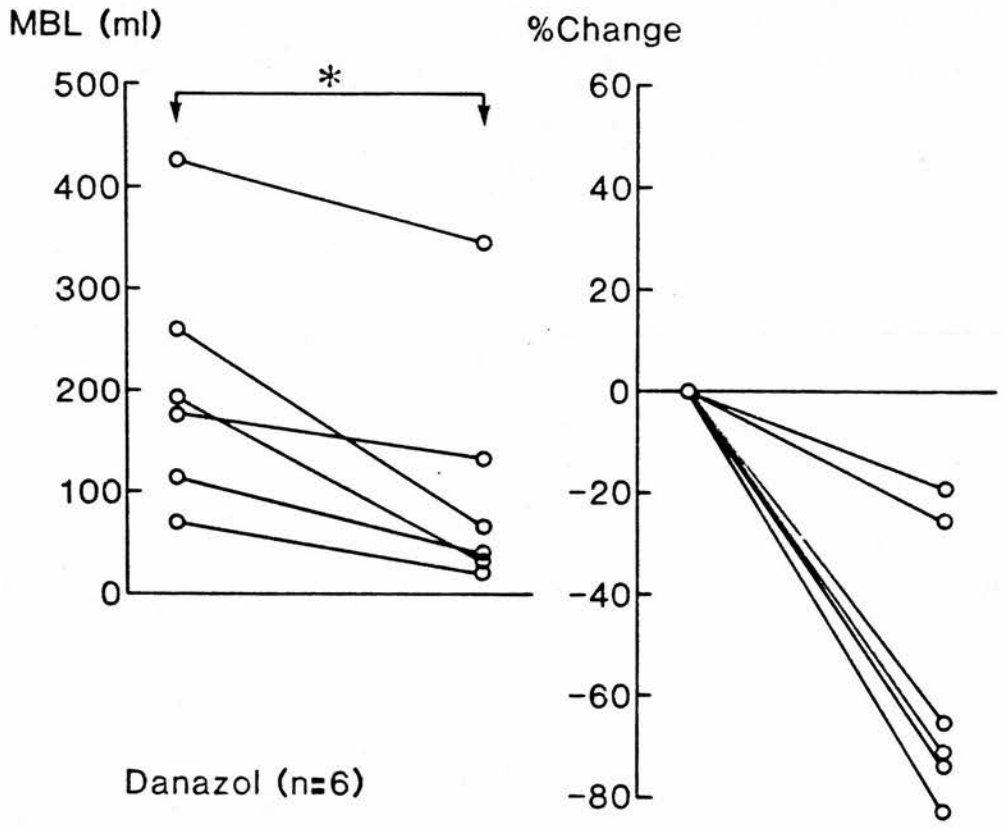
Table 2(ii) 2 shows the blood loss and menstrual cycle data for the 2 control cycles of the 4 treatment groups. There was no

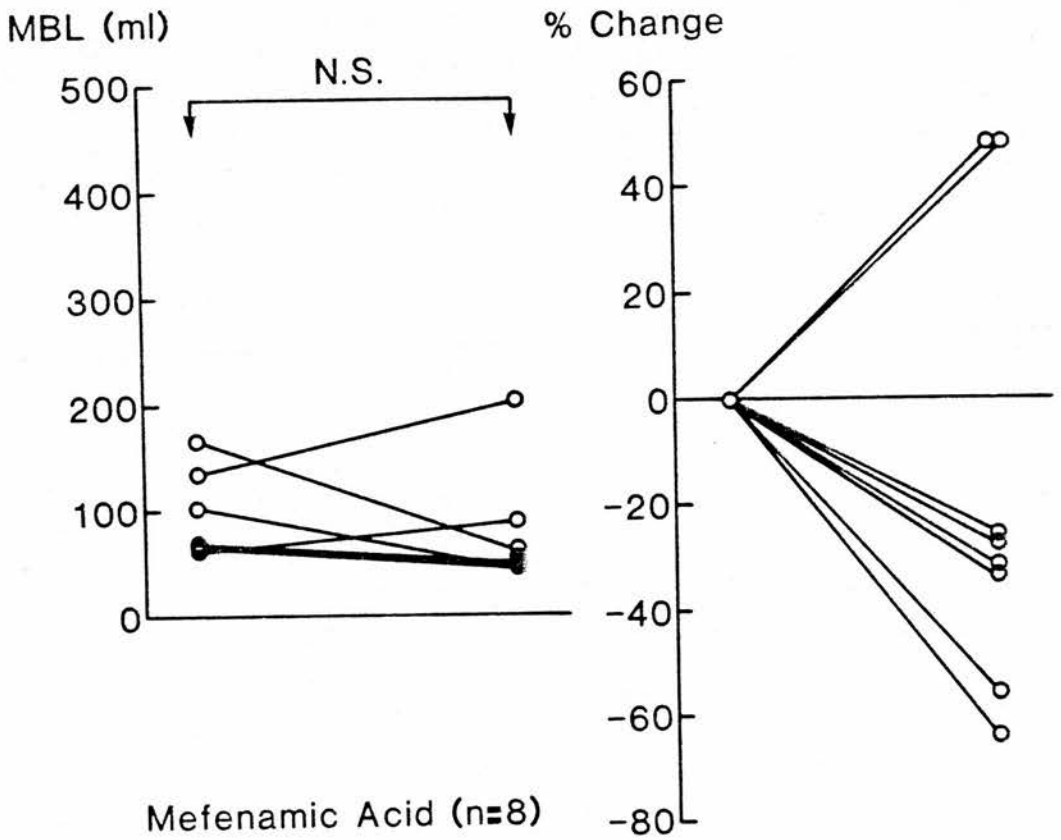
difference in the number of days bleeding or the interval between periods, but those women who received danazol had a heavier blood loss pre-treatment than those women in the mefenamic acid or the progesterone coil groups.

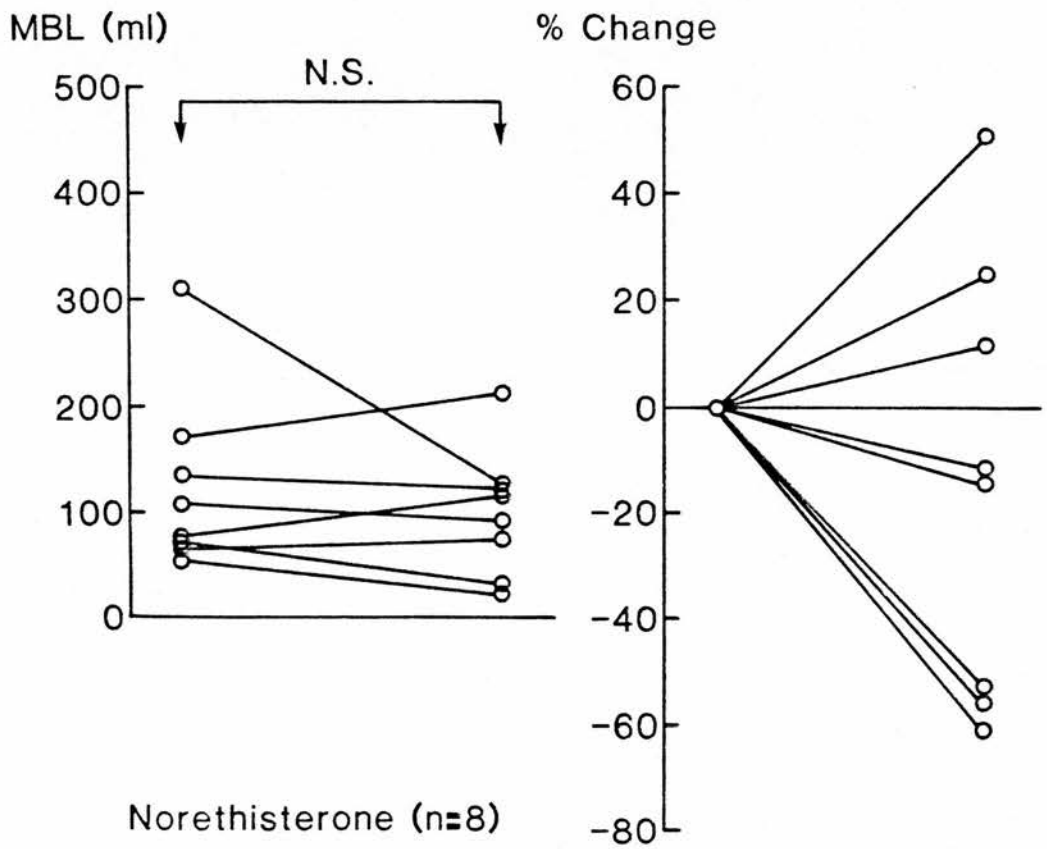
TREATMENT(n)	MBL(mls)	DURATION(days)	CYCLE LENGTH(days)
DANAZOL(6)	[ab]187(70,429)	6(3,7)	29(24,31)
MEFENAMIC ACID(8)	[a]68(61,169)	5(4,7)	28(23,38)
NORETHISTERONE (8)	94(55,312)	6(4,7)	28(24,30)
PROGESTERONE COIL(8)	[b]71(56,164)	5(4,6)	26(23,30)

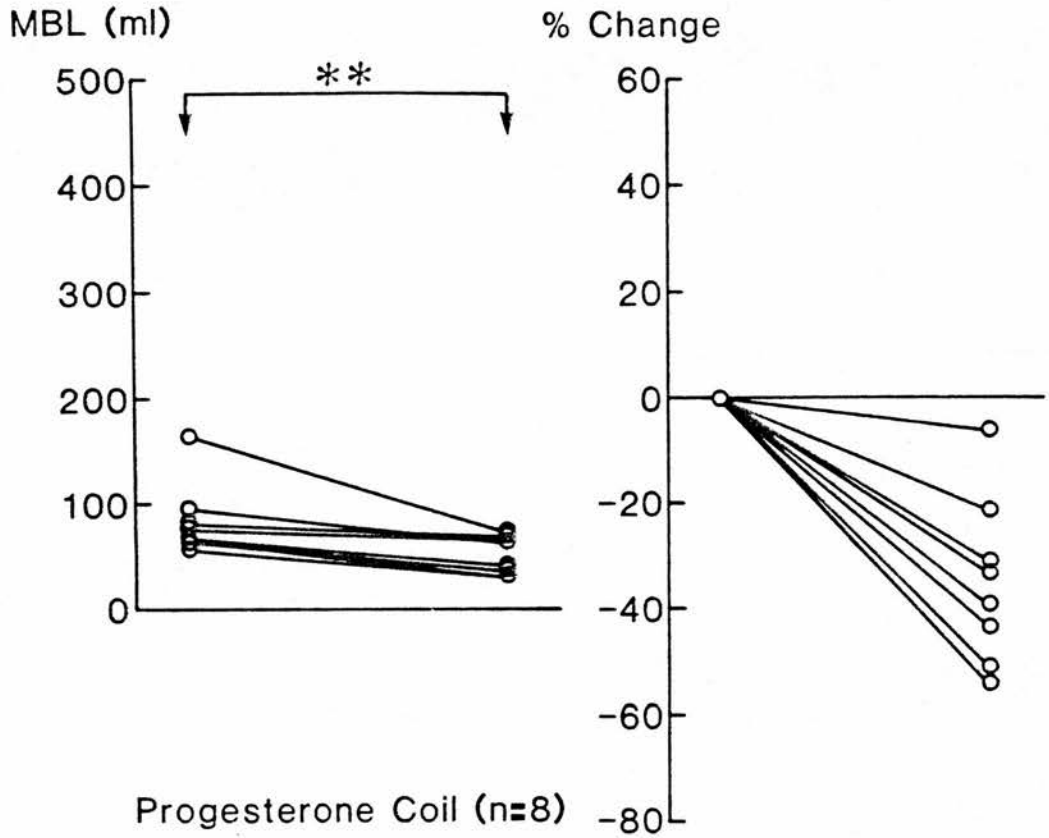
Table 2(ii)2 Blood loss and menstrual cycle characterics. Median values are given with the range in brackets. aa and bb : p = 0.01

The effects of medical treatment on MBL are presented in Figures 2(ii)1 to 2(ii)4. In the danazol, norethisterone and progesterone coil groups the treatment results represent the mean blood loss of the last 2 cycles (i.e. cycles 4 and 5), whereas in the mefenamic acid group the mean loss over cycles 3 and 4 is given. Changes in blood loss are shown in absolute figures, and as a percentage of the original blood loss. Treatment with danazol and the progesterone coil resulted in a significant fall in MBL. There was no overall improvement in blood loss with either norethisterone or mefenamic acid - however in the latter group the loss was reduced by between 24% and 62% in 6 of 8 cases. These data are again


MBL (ml)
%Change
*
500
400
300
200
100
0
60
40
20
0
-20
-40
-60
-80
Danazol (n=6)


MBL (ml)
% Change
N.S.
500
400
300
200
100
0
60
40
20
0
-20
-40
-60
-80
Mefenamic Acid (n=8)


MBL (ml)
% Change
N.S.
500
400
300
200
100
0
60
40
20
0
-20
-40
-60
-80
Norethisterone (n=8)


MBL (ml)
% Change
**
500
400
300
200
100
0
60
40
20
0
-20
-40
-60
-80
Progesterone Coil (n=8)

displayed in table 2(ii)3 along with the number of days bleeding and the cycle length before and after treatment. Apart from a doubling in the median duration of bleeding after the insertion of the progesterone coil, there were no other differences between the groups.

TREATMENT(n)		MBL(mls)	DURATION(days)	CYCLE(days)
DANAZOL(6)	Pre	*187(70,429)	6(3,7)	29(24,31)
	Post	* 54(30,347)	4(2,6)	28(26,29)
MEFENAMIC ACID(8)	Pre	68(61,169)	5(4,7)	28(23,38)
	Post	51(45,203)	5(4,7)	28(27,36)
NORETHISTERONE(8)	Pre	94(55,312)	6(4,7)	28(24,30)
	Post	106(24,216)	5(3,7)	28(26,29)
PROGESTERONE	Pre	** 71(56,164)	** 5(4,6)	26(23,30)
COIL (8)	Post	** 55(31,75)	** 10(7,13)	26(24,37)

Table 2(ii) 3. MBL and menstrual cycle data before (pre) and after (post) treatment. *p<0.05, **p<0.01.

The median haemoglobin concentration, white cell, platelet and reticulocyte counts did not differ between the 4 groups pre-treatment, and no significant changes occurred after treatment itself.

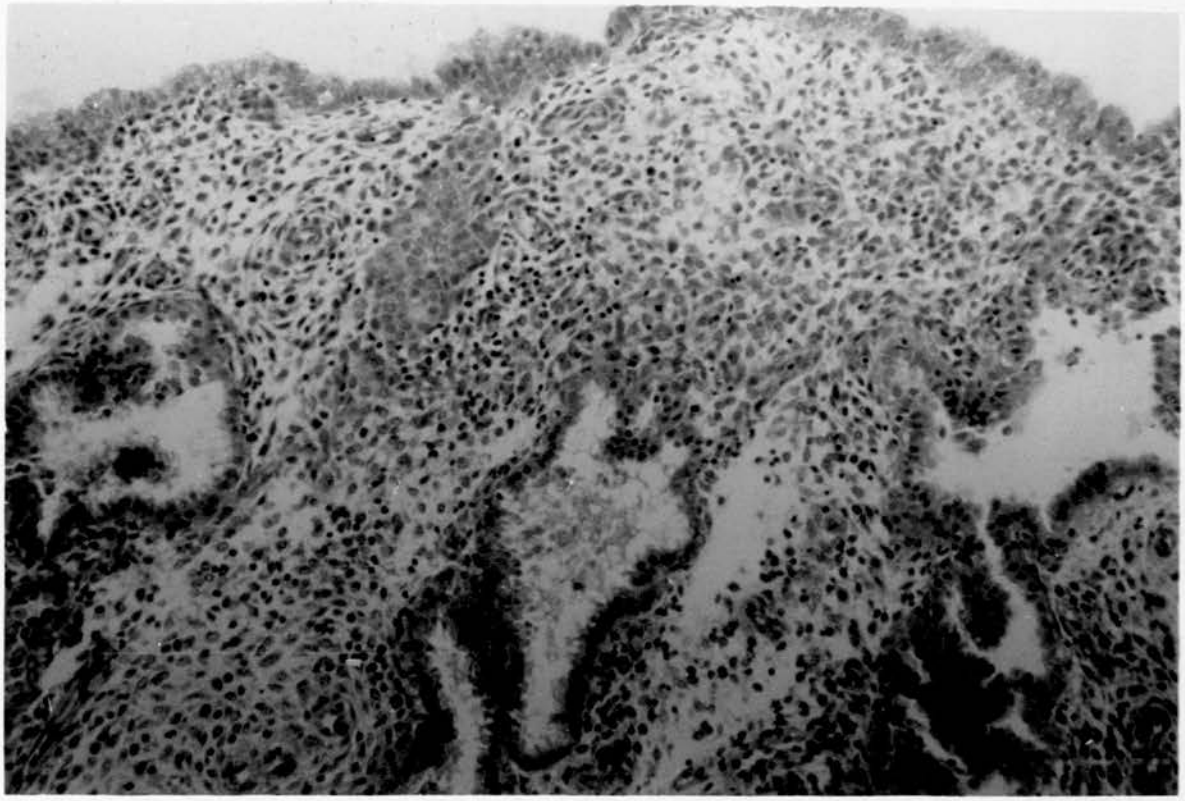
All 30 women had ovulated in their first 2 cycles, with a urinary pregnanediol: creatinine ratio > 1mg/gm, or a serum progesterone concentration > 18nmol/L, in the mid luteal phase (5-12 days prior

to the subsequent period). In addition those women who had undergone endometrial biopsy, and in whom sufficient endometrium had been obtained, showed histological evidence of secretory activity in keeping with their menstrual dates. Table 2(ii) 4 shows the serum estradiol and progesterone concentrations of the 4 treatment groups in the mid luteal phase of the 4th (mefenamic acid) or 5th (danazol, norethisterone, progesterone coil) study cycles. The number of women in each group showing presumptive evidence of ovulation was 1(20%), 7(88%), 2(29%) and 4(67%) in the danazol, mefenamic acid, norethisterone and progesterone coil groups respectively. The occurrence of ovulation did not differ statistically between the 4 groups ($p > 0.05$, Fisher exact probability test).

PATIENT	DANAZOL		MEFENAMIC ACID		NORETHISTERONE		PROGESTERONE COIL	
NO	E2	P4	E2	P4	E2	P4	E2	P4
1	163	4.6	486	80.4	86	<2.1	471	35.2
2	168	19.5	397	43.2	83	2.6	379	35.4
3	111	<2.1	228	6.6	94	<2.1	463	8.6
4	99	<2.1	401	39.6	101	6.4	187	<2.6
5	167	<2.1	388	20.6	154	33.6	696	39.6
6	-	-	312	21.4	232	29.0	542	55.7
7			506	19.7	<70	<2.1	-	-
8			826	59.1	-	-	-	-
MEDIAN	163	<2.1	399	30.5	94	2.6	467	35.3

Table 2(ii)4. Endocrine parameters after treatment. Individual values for serum estradiol (E_2: pmol/L) and progesterone (P_4:nmol/l) are given. Samples not obtained between 5 and 12 days prior to the subsequent menstrual period have been excluded.

The histological analysis of the endometrial biopsies is summarised in table 2(ii) 5. The 6 mefenamic acid-treated women all showed a histological picture in keeping with their dates (figure 2(ii)5) whereas this was only true of one woman in the progesterone coil



group. The remainder of these women showed a mixed picture of inactive glandular elements with pseudodecidualisation (figure 2(ii)6). In the danazol-treated group, the endometrium was scanty; samples for histological assessment were obtained from 3 women in only one of whom were secretory changes detected.

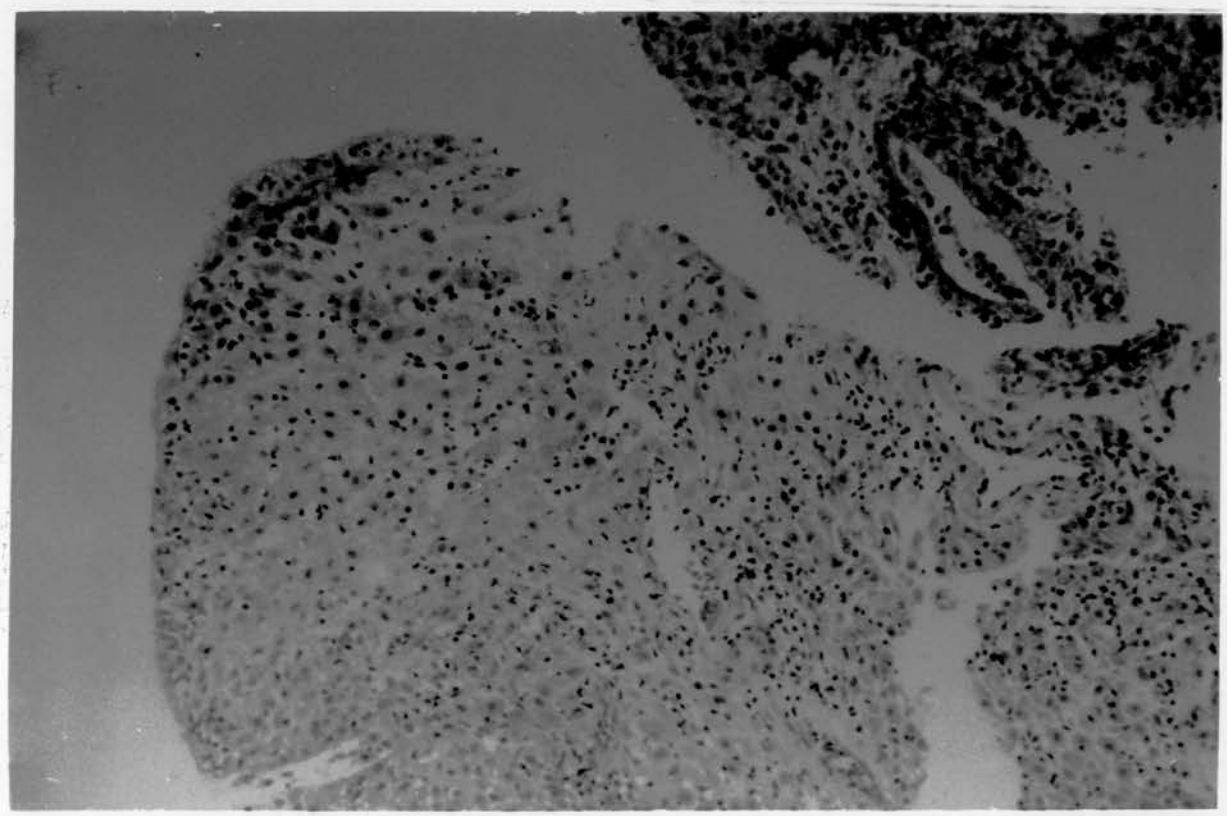
PATIENT NO	DANAZOL	MEFENAMIC ACID	NORETHISTERONE	PROGESTERONE COIL
1	P	S	ES	S
2	S	S	S	P + ES + D
3	P	S	S	I + D
4	N	N	S	P + ES + D
5	N	S	N	N
6	N	S	ES	ES + D
7		N	P	N
8		S	S	I

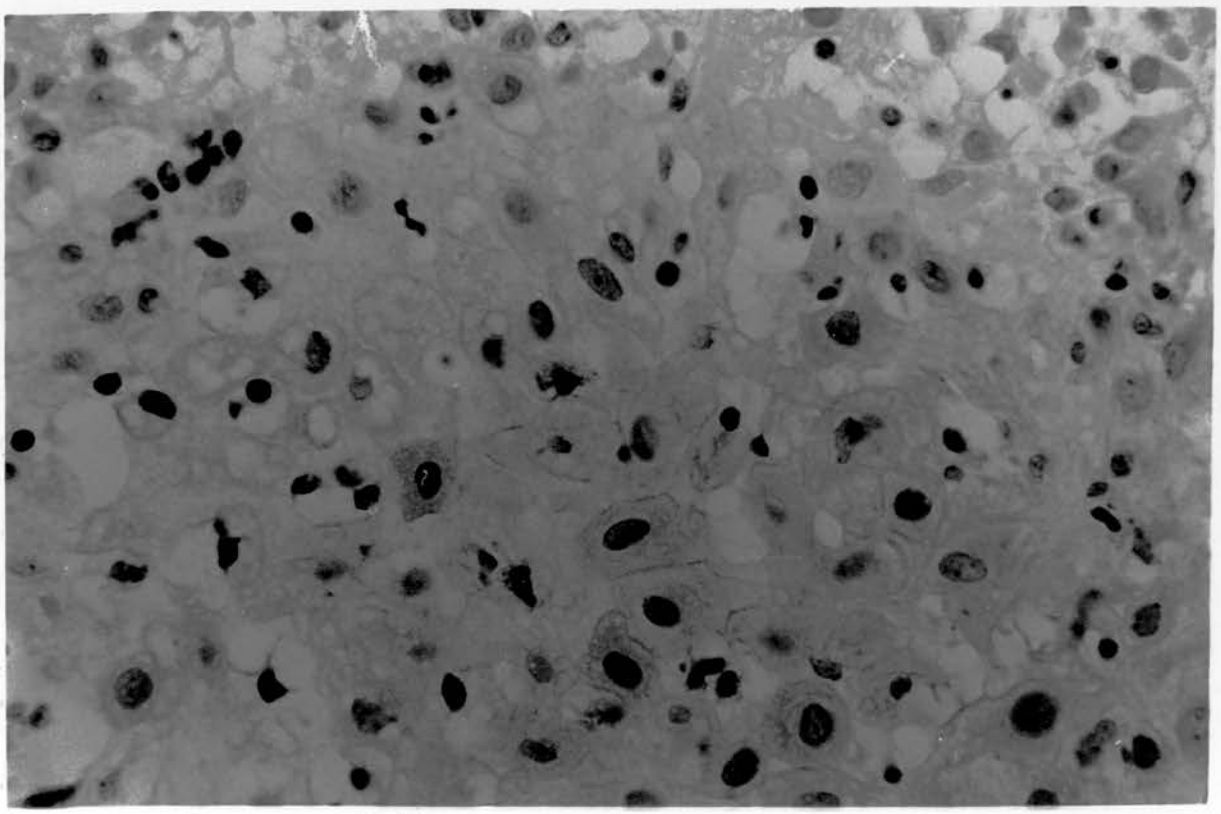
Table 2 (ii)5. Endometrial histology. Reports from individual patients are summarised (P - proliferative: ES - early secretory change less advanced than menstrual dates: S - secretory change in keeping with dates: I - inactive glands: D-pseudodecidualisation: N - no biopsy obtained or insufficient tissue for histological assessment).

Finally no patient complained of serious side effects with any form of treatment, and none chose to withdraw from medical treatment once enrolled in the study.

DISCUSSION

Using the alkaline haematin method it has been possible to objectively assess the efficacy of 4 commonly used agents for the





treatment of menorrhagia. Although other treatment methods are available, such as the combined contraceptive pill (Nilsson & Rybo, 1971), fibrinolytic inhibitors (Ylikorkala & Viinikka, 1983) and ethamsylate (Harrison & Campbell, 1976), mefenamic acid, norethisterone and the progesterone-impregnated coil were chosen to assess their effects on endometrial PG production (see Chapter 3), and danazol was selected as a treatment method not necessarily thought to act via changes in endometrial prostanoids.

Each patient acted as her own control, and although treatment was allocated randomly, this was neither blind, nor placebo controlled. Previous workers have failed to show a significant improvement in MBL with placebo (Chimbira et al, 1980), however between group comparisons in the present study should be interpreted with caution.

The 4 groups were similar, but the pre-treatment loss in the women randomised to the danazol group was significantly greater than that of those women treated with either mefenamic acid or the progesterone device. Due to the small numbers this was unavoidable, but the finding should be borne in mind, for it has been demonstrated that some methods of medical treatment are more effective with a higher pre-treatment blood loss (Guillebaud et al, 1978). Despite this increased MBL in the danazol group though, there were no corresponding differences in haematological indices.

All individuals were selected to ensure that they exhibited ovulatory cycles during the 2 control months. This is of particular importance when considering the therapeutic effects of

drugs such as norethisterone, which has been advocated as a logical treatment method in cases of anovulation to prevent metropathia haemorrhagica (Bishop & Almeida, 1960). In addition, as the majority of women with menorrhagia have ovulatory dysfunctional uterine bleeding (see Chapter 2(i)), it is pertinent to examine the effects of treatment on this group specifically.

The MBL after treatment has been calculated as the mean loss of the 3rd and 4th cycles following mefenamic acid, or the 4th and 5th cycles following the other forms of treatment. This was necessary to ensure that the "treatment periods" were the result of adequate drug exposure, for although the PG synthetase inhibitor mefenamic acid was taken during the 3rd period itself, the administration method of the other drugs would lead to an expected treatment effect in the 4th and subsequent periods.

Blood loss was significantly improved by treatment with danazol or the progesterone coil, with all the women in these groups having a reduced loss in their treatment cycles. Although the overall loss did not change in the other 2 groups, there was a wide variation, with 6(76%) of the mefenamic acid group and 5 (63%) of the norethisterone group showing some improvement. The interval between periods was unaffected by treatment, but all 8 women treated with the medicated coil experienced inter-menstrual spotting, and the median duration of the period itself was doubled.

The results of the danazol, mefenamic acid and progesterone coil groups are in accord with previously reported studies. An improvement in both MBL and haemoglobin concentration was seen using danazol 400mg daily (Chimbira et al, 1979) but this was also

associated with a significant decrease in the duration of bleeding (7.7 ± 0.8 days to 3.6 ± 0.5 days) after 2 months of treatment.

However, this high dose of drug resulted in two of the 18 women withdrawing from study because of side effects (acne, muscle cramps and weight gain). Subsequent studies investigating different dose regimens (400, 200 and 100 mg) confirmed the effectiveness of 200 mg daily with a reduced incidence of side effects (Chimbira et al, 1980). Both the degree of blood loss and the number of days bleeding were reduced, though as with the present study, there was no change in cycle length. The mechanism of action of danazol in menorrhagia appears to be via both an effect on the hypothalamo-pituitary-ovarian axis and a direct effect at the level of the endometrium itself. In this work, a luteal phase progesterone concentration consistent with ovulation was only seen in 1 of 5 women, which is in keeping with the results of previous studies using 200mg of the drug daily (Colle and Greenblatt, 1976; Van Dijk et al, 1979). Ovulation inhibition appears to be less consistent with lower doses of danazol, however Greenblatt et al (1974) noted a 94% anovulation rate in 38 of 46 women receiving 100 mg daily, although cyclical menstrual periods continued to occur.

The 25% overall reduction in MBL with mefenamic acid seen in this study (68 mls to 51 mls - median values) agrees well with the results of others (Fraser et al, 1981: 28% - Ylikorkala & Viinikka, 1983: 24% - Dockeray et al, 1986: 20%), although earlier work from Oxford (Anderson et al, 1976) suggested a 50% overall improvement in MBL in 6 women. Such data have to be interpreted with care in relation to differing patient characteristics, variations in the duration and dosage of treatment, differences in the underlying

cause of the excessive blood loss, and variations in the initial degree of blood loss itself (Guillebaud et al, 1978; Fraser et al, 1981). In 2 (25%) cases in the present work there was a deterioration in blood loss of 48%. Such failure to respond to the cyclooxygenase inhibitor has been noted elsewhere, with 15 (22%) women in one study showing no objective improvement in MBL with mefenamic acid (Fraser et al, 1981). It may be that in some individuals endometrial PGs do not play a major role in the control of blood loss. Alternatively, the concentration of mefenamic acid itself may fail to reach adequate levels at its site of action in some cases - for it has been shown that there is a direct positive relationship between peripheral mefenamic acid concentrations and improvements in the degree of MBL (Dockeray et al, 1986). Furthermore, it is possible that the abnormal bleeding in such "non-responders" to mefenamic acid could be the result of augmented leukotriene synthesis following inhibition of the cyclooxygenase pathway (Hahn et al, 1985; Rees et al, 1986).

Mefenamic acid is thought to act via a local mechanism on uterine prostanoids, without affecting the hypothalamo-pituitary-ovarian axis. This will be considered further in Chapter 3, and would be supported by the endocrine and histological data presented in tables 2(ii)4 and 2(ii)5.

A significant improvement in blood loss with the progesterone-impregnated coil has also been reported by others (Troughbough et al, 1978; Berqvist & Rybo, 1983), and as in the present case this treatment has been associated with the occurrence of menstrual spotting and prolongation of the period. This

disturbance in the menstrual pattern though must be seen balanced against the advantages of an effective treatment method which obviates the need for repeated oral drug administration. In addition the reduced dosage employed for such local therapy should decrease the incidence of unpleasant systemic side effects.

The main action of the progesterone coil appears to be local, and most studies have shown endometrial glandular suppression, with pseudodecidualisation, in the absence of disturbances of the hypothalamo-pituitary-ovarian axis (Bryant-Greenwood et al, 1977; Hagenfeldt et al, 1977; Bonnar & Shephard, 1979; Shaw et al, 1981). The present data would support this, with 4 (67%) cases showing a luteal phase increase in serum progesterone compatible with ovulation, and with only one of 6 endometrial biopsies revealing a histological picture in keeping with the menstrual dates. The wide variation of histological appearances actually seen though probably reflects the relatively short duration (2-3 months) of coil exposure (Hagenfeldt et al, 1977).

The effects of the treatment of abnormal MBL with norethisterone have not been objectively assessed previously, although Bishop & Almeida (1960) did report a <u>subjective</u> improvement in blood loss in 34 of 52 cycles in 13 women with ovulatory dysfunctional bleeding. Here, there was no consistent effect on MBL, and in only 2 (29%) cases was there a rise in the mid luteal progesterone concentration >18nmol/L. However this would not necessarily indicate an inhibition of ovulation , for Larsson-Cohn et al (1971) have suggested that gestogen administration itself may cause a defect in corpus luteum function.

The predominantly secretory change seen in the endometrial biopsies could be the result of either ovulation, or a direct effect of the gestogen on the endometrium.

Along with mefenamic acid, norethisterone is used widely for the medical treatment of menorrhagia; the results of the present study would question its value as a first line therapy in women with ovulatory cycles.

CHAPTER 3

THE RELATIONSHIP BETWEEN ENDOMETRIAL PROSTAGLANDINS AND MENSTRUAL BLOOD LOSS, and

THE EFFECTS OF MEDICAL TREATMENT FOR MENORRHAGIA ON ENDOMETRIAL PROSTAGLANDIN CONCENTRATIONS

The suggested relationship between endometrial prostaglandin concentrations and the degree of MBL outlined in Chapter 1 will now be examined in more detail. In addition the effects on endometrial PG synthesis of the four medical treatments for menorrhagia described in Chapter 2 will be assessed. As stated, these drugs were chosen to observe, (i) the effect of PG synthetase inhibition (mefenamic acid) on endometrial prostanoid production, (ii) the influence of both natural progesterone (progesterone-impregnated coil) and the synthetic gestogen, norethisterone, on endometrial PGs, and (iii) the actions, if any, of another agent (danazol), which, though effective as a treatment for menorrhagia is not thought to act via the arachidonic acid cascade.

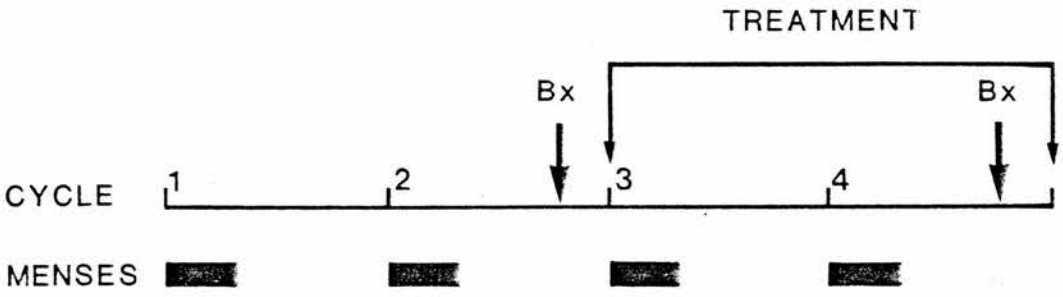
PATIENTS AND METHODS

The objective assessment of menorrhagia in 70 women with a subjective complaint of heavy menses has been described in Chapter 2. Of these, 34 individuals with ovulatory cycles agreed to undergo endometrial biopsies in the mid-luteal phase of their second study cycle. 30 women with a measured blood loss >50 mls were subsequently treated with one of the four medical therapies, and a further biopsy was obtained in the mid-luteal phase of the second or third treatment cycle (figure 3.1).

Endometrial biopsies were taken using a Sharman curette without anaesthesia.

Tissue Collection and prostaglandin measurement

Biopsies were collected on ice into modified 199 medium (Flow


TREATMENT
Bx
Bx
CYCLE
1
2
3
4
MENSES

Laboratories, U.K.). After transport to the laboratory within 5 minutes, the tissue was blotted dry on a gauze swab, weighed, and immediately placed in ice-cold ethanol. The tissue was then homogenised using a polytron homogeniser, and centrifuged at 1500g for 10 minutes at 4°C. The supernatant was aspirated and dried under nitrogen.

Samples were oximated overnight at 20°C and prostaglandin concentrations were determined using radioimmunoassay with specific antisera raised against PG methyloximes (Mox) (Kelly et al, 1986a). Separation of free and bound moieties was effected using polyethylene glycol 6000. Cross-reactivities, determined by the amount of PG that caused 50% inhibition of binding of the appropriately-labelled PG were as follows: $PGF_{2\alpha}$ antiserum with $PGF_{1\alpha}$, 7.2%; $PGF_{3\alpha}$, 2.9%; $PGF_{2}\beta$, 3.5%; PGE_2, 1.1%; 6oxo$PGF_{1\alpha}$, 1.1%; 13,14 dihydro$PGF_{2\alpha}$, 1.0%; all other PGs tested, <0.2%: PGE_2(Mox) antiserum with PGE_1, 53%; PGE_3, 31%; PGB_2, 0.2%; 15 oxo PGE_2, 0.25%; 20-hydroxyPGE_2, 3.7%; 8-iso PGE_2, 2.9%: 6oxo$PGF_{1\alpha}$ (Mox) antiserum with 13,14 dihydro 6oxo$PGF_{1\alpha}$, 13.3%; 6oxoPGE_1, 9.1%; 6,15 dioxo $PGF_{1\alpha}$, 0.25%; 13,14 dihydro $PGF_{2\alpha}$, 0.23%; all other PGs tested, <0.2%. Due to the cross reactivity of PGE_2 with other PGs, the value for the concentration of PGE subsequently quoted will potentially include prostaglandin from the 1,2 (and 3) series.

The assay sensitivity (defined as the amount of PG distinguishable from zero with 95% confidence limits) was 2pg. The inter-and intra-assay coefficiants of variation for 6oxo$PGF_{1\alpha}$, PGE and $PGF_{2\alpha}$ were 9% and 7%, 13% and 12%, and 14% and 11% respectively (n = 19 and 15 for each PG).

Validation of Methods

i) Resuspension studies

In 5 cases, following the aspiration of the supernatant during the PG extraction, the residual pellet was resuspended in ethanol and re-centrifuged 3 times, to assess the efficiency of the extraction technique.

ii) Representative studies

Endometrium was obtained at diagnostic curettage from 5 women with non malignant disease from 3 different areas of the uterus (fundus, anterior and posterior walls). PG concentrations were assessed as already described to determine the representative nature of isolated endometrial biopsies.

Endometrial dating

In all experiments, a portion of endometrium was placed in formol saline for histological dating (Noyes et al, 1950).

RESULTS

Resuspension studies

The median (range) percentage PG recovery with the initial ethanol extraction was 91% (81,85), 84% (83,92) and 96% (81,97) for 6oxoPGF$_{1\alpha}$, PGE and PGF$_{2\alpha}$ respectively (n = 5).

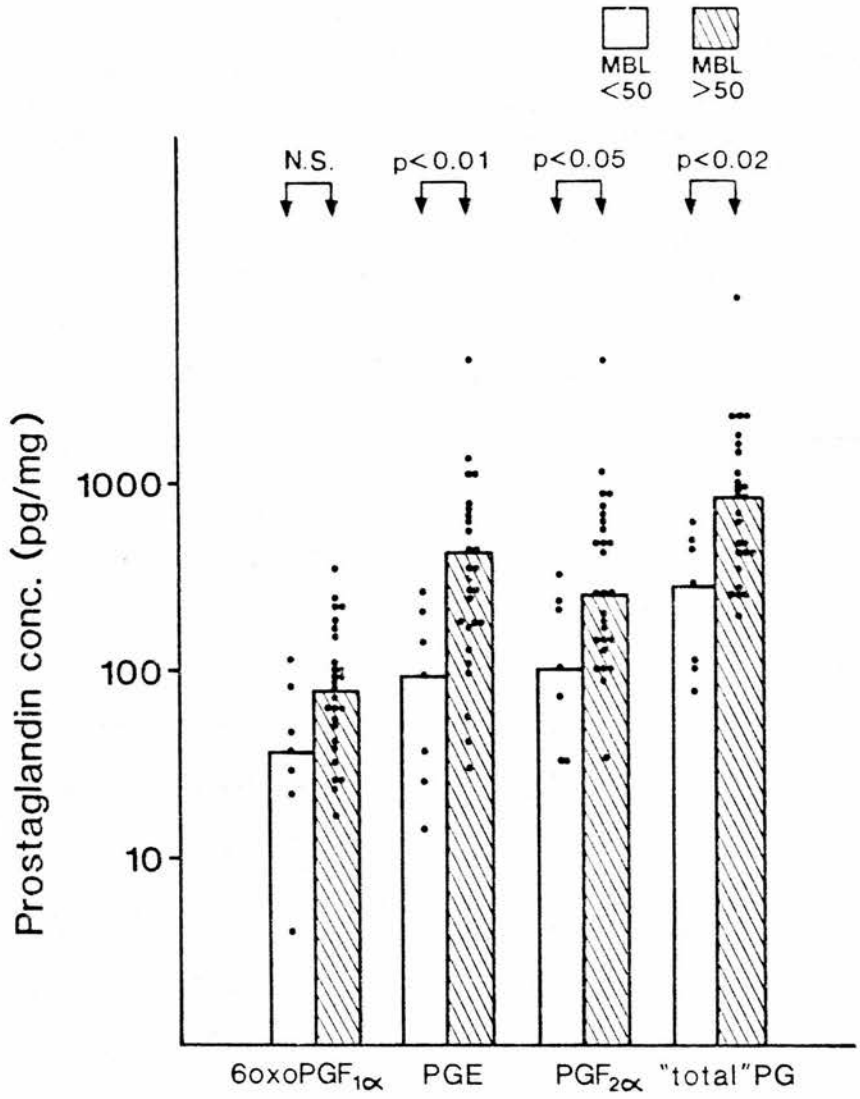
Representative studies

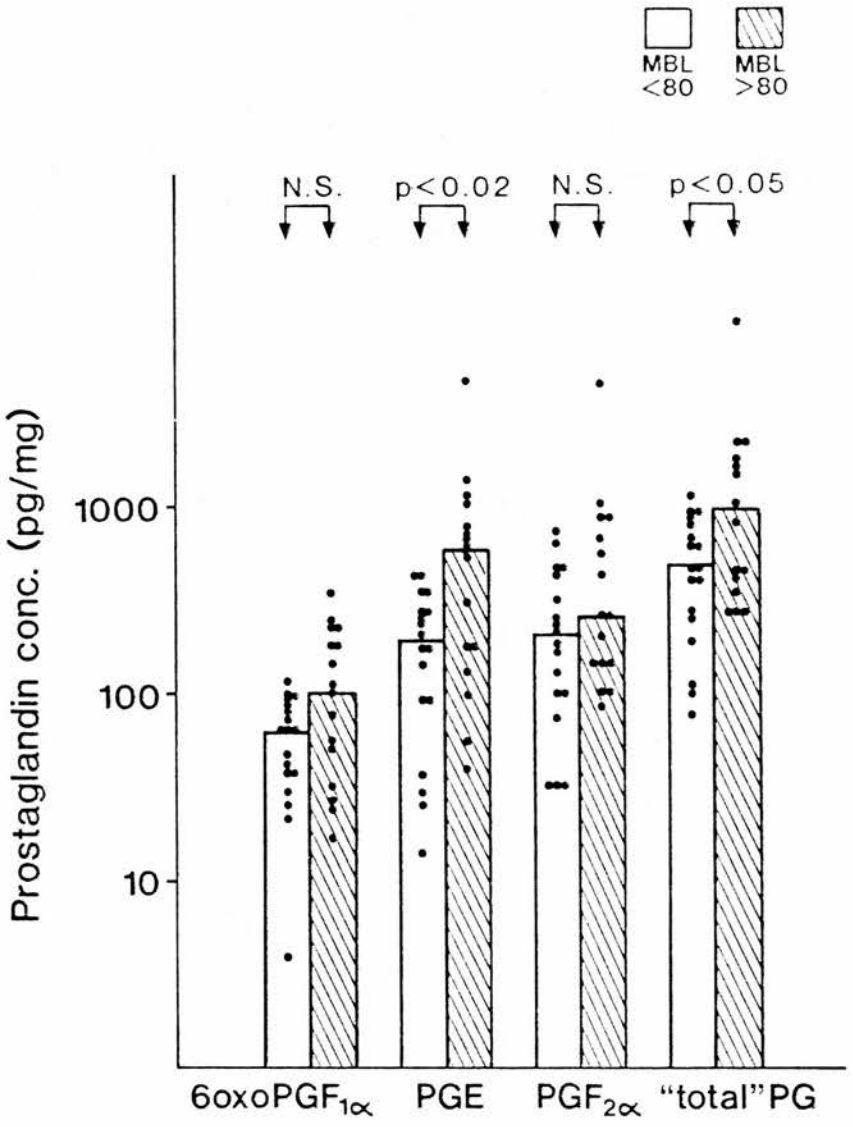
The median (range) coefficient of variation for the 3 endometrial biopsy specimens was as follows: 6oxo$PGF_{1\alpha}$ - 16% (7,22); PGE-15% (9,18), and $PGF_{2\alpha}$-16% (11,18); n = 5.

Endometrial prostaglandin concentrations and menstrual blood loss

The relationship between endometrial PG concentrations and measured MBL is shown in figures 3.2 and 3.3. There was a wide range of observed endometrial prostanoid concentrations. In those women with a blood loss of <50 mls (34 mls (range 18,40)- n = 7) the endometrial concentrations of PGE, $PGF_{2\alpha}$ and "total PG" (PGE+$PGF_{2\alpha}$+6oxo$PGF_{1\alpha}$) were less than those in the endometrium of women with heavy menses (MBL 98mls (range 55, 432)- n = 27). The concentrations of PGE and $PGF_{2\alpha}$ did not differ in either group of women, but although the concentration of 6oxo$PGF_{1\alpha}$ was significantly less than that of PGE or $PGF_{2\alpha}$ in those women with heavy menses (MBL >50mls), there was no difference between the endometrial concentrations of the 3 PGs in those women with a blood loss of <50 mls.

If the results are analysed comparing women with a blood loss of greater or less than 80 mls, a similar pattern emerges. Again the endometrial concentrations of PGE and $PGF_{2\alpha}$ did not differ in either group of women, however there was no significant increase in $PGF_{2\alpha}$ in those women with heavy menses (MBL = 152 mls (86,432), n = 16) in relation to those with a "normal" blood loss (59 mls


MBL <50
MBL >50
N.S.
p<0.01
p<0.05
p<0.02
Prostaglandin conc. (pg/mg)
1000
100
10
6oxoPGF1α
PGE
PGF2α
"total"PG


MBL <80
MBL >80
N.S.
p<0.02
N.S.
p<0.05
Prostaglandin conc. (pg/mg)
1000
100
10
6oxoPGF1α
PGE
PGF2α
"total"PG

(18,78), n = 18). In both groups of women the concentration of 6oxoPGF$_{1\alpha}$ was significantly less than that of PGE or PGF$_{2\alpha}$. These data are summarised numerically in table 3.1.

I	<50mls(7)		>50mls(27)
MBL(mls)	34(18,40)	p<0.001	98(55,432)
PGE	92(14,266)	p<0.01	420(30,4604)
PGF$_{2\alpha}$	100(33,326)	p<0.05	254(35,4554)
		***	
6oxoPGF$_{1\alpha}$	37(4,113)	***	79(17,348)
TOTAL PG	272(78,614)	p<0.02	818(193,9506)
II	<80mls(18)		>80mls(16)
MBL(mls)	59(18,78)	p<0.001	152(86,432)
PGE	190(14,422)	p<0.02	583(40,4604)
	*		*
PGF$_{2\alpha}$	202(33,723)		257(88,4554)
	**		*
6oxoPGF$_{1\alpha}$	62(4,113)		102(17,348)
TOTAL PG	481(78,1104)	p<0.05	952(265,9506)

Table 3.1 The relationship between menstrual blood loss (MBL) and endometrial PG concentrations (pg/mg) for women with a blood loss greater or less than 50 mls (I), or 80 mls (II) respectively. All data represent the median (range). Between group statistical analysis is represented in the central column. Asterisks refer to statistical differences between prostaglandin concentrations in any one group of women (* - p<0.01: ** - p<0.002: *** - p<0.001).

The effects of medical treatment on endometrial prostaglandin concentrations

The clinical response to danazol, mefenamic acid, norethisterone and the progesterone-impregnated coil has been described in Chapter 2. Of the 30 women, satisfactory biopsies were obtained both before and after treatment on 23 occasions. The changes in MBL and endometrial PGs are presented in Table 3.2 and figures 3.4-3.7.

Danazol

MBL was improved in all four cases in which endometrial biopsies were obtained, however the effects on endometrial PG production are difficult to interpret due to the small number of cases. Biopsies were attempted in the remaining two patients, however, the degree of endometrial atrophy prevented adequate tissue sampling.

Mefenamic acid

There was an overall improvement in the degree of MBL, but this was not associated with changes in endometrial PG concentrations. Of the 8 women treated with mefenamic acid, one declined to undergo biopsy post-treatment, and in another, inadequate tissue was obtained.

Progesterone-impregnated coil

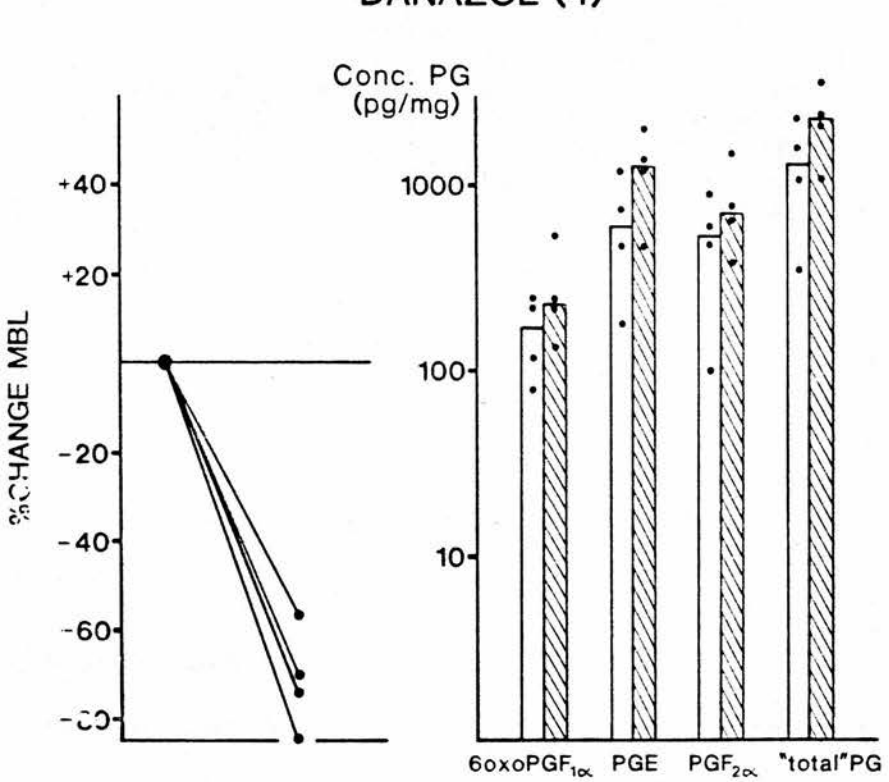
The improvement in MBL in all 7 cases was associated with a significant reduction in PGE, $PGF_{2\alpha}$ and "total" endometrial PG concentrations. Insufficient tissue was obtained for assay in one case.

	CONTROL					TREATMENT				
	MBL(mls)	6oxoPGF$_{1\alpha}$	PGE	PGF$_{2\alpha}$	"total" PG	MBL(mls)	6oxoPGF$_{1\alpha}$	PGE	PGF$_{2\alpha}$	"total"PG
DANAZOL (4)	203(70,261)	172(79,243)	593(175,1184)	535(100,838)	1299(354,2265)	51,(20,77)	228(134,529)	1210(466,1926)	694(365,1413)	2235(1066,3560)
MEFENAMIC ACID (6)	a 85(68,169)	60(17,348)	186(30,4604)	178(88,4554)	412(256,9506)	a 47(39,210)	64(23,173)	246(192,2388)	234(159,873)	546(412,3434)
PROGESTERONE COIL (7)	b 64(56,164)	87(25,180)	c 278(174,767)	d 450(130,683)	e 842(265,1630)	b 45(31,77)	55(26,124)	c 100(26,418)	d 160(38,317)	e 273(178,832)
NORETHISTERONE (6)	131(55,259)	69(26,232)	220(40,1379)	232(34,1017)	770(152,2251)	110(24,222)	110(18,187)	403(13,1080)	455(24,720)	985(55,1987)

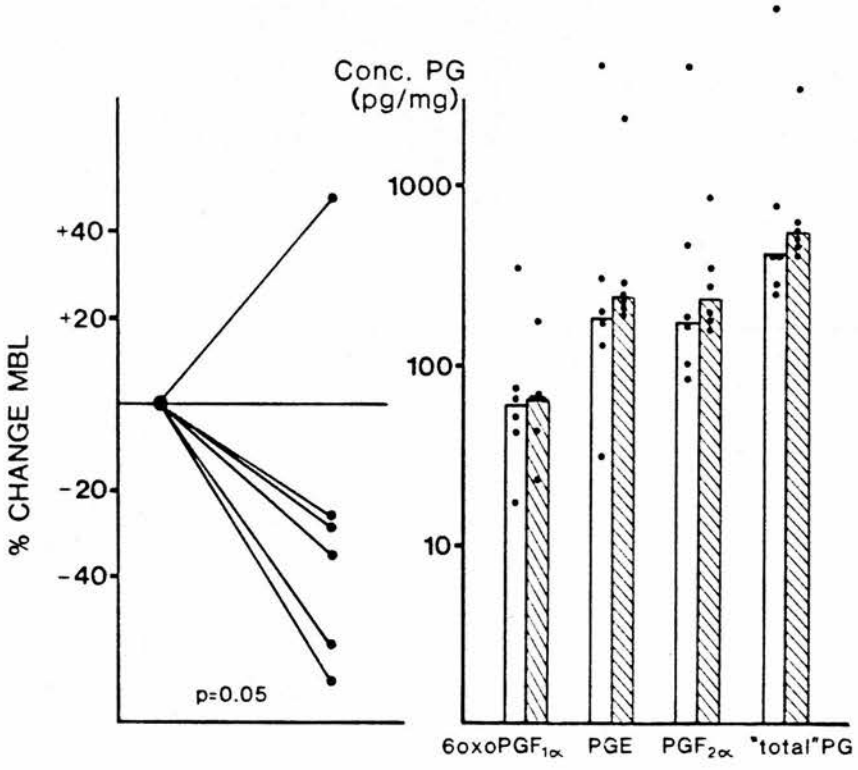
Table 3.2 The relationship between menstrual blood loss (MBL) and endometrial PG concentrations (pg/mg) before and after medical treatment. All data represent the median (range).

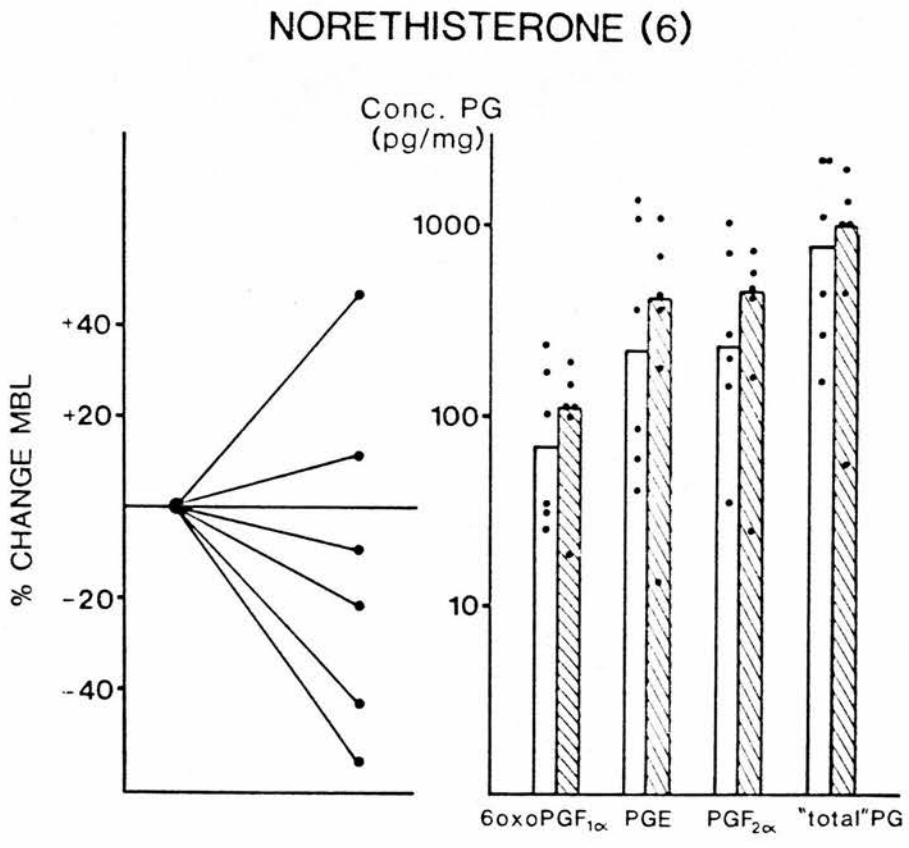
aa - $p = 0.05$, Wilcoxon rank sum test ($p > 0.05$, Wilcoxon signed rank test): bb - $p < 0.05$, Wilcoxon signed rank test: cc - $p = 0.05$, Wilcoxon signed rank test: dd - $p = 0.05$, Wilcoxon signed rank test: ee - $p = 0.05$, Wilcoxon rank sum test ($p > 0.05$, Wilcoxon signed rank test).

Analysis of blood loss between groups has been discussed in Chapter 2(ii).

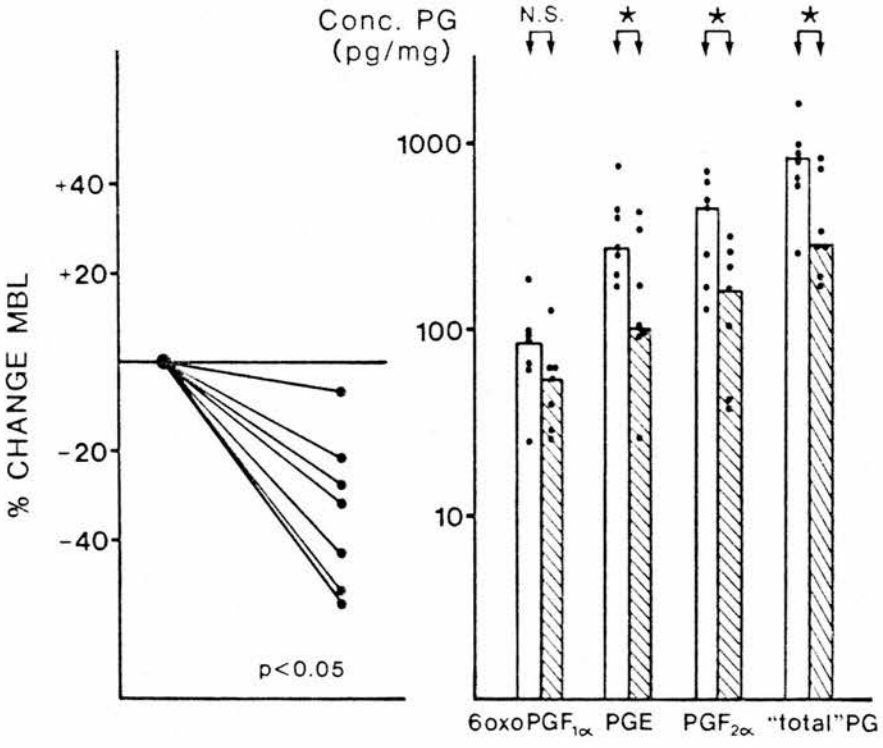

DANAZOL (4)
Conc. PG
(pg/mg)
%CHANGE MBL
+40
+20
-20
-40
-60
1000
100
10
6oxoPGF1α
PGE
PGF2α
"total"PG

MEFENAMIC ACID (6)




NORETHISTERONE (6)
% CHANGE MBL
+40
+20
-20
-40
Conc. PG
(pg/mg)
1000
100
10
6oxoPGF1α
PGE
PGF2α
"total"PG

PROGESTERONE COIL (7)



Norethisterone

There were no significant changes in endometrial PG concentrations after norethisterone therapy. 2 patients failed to attend for their second biopsies - MBL had improved following treatment in one case, and worsened in the other.

DISCUSSION

The conflict in the reported association between endometrial PGs and menorrhagia has been pointed out in Chapter 1. Many studies have shown a relationship between the vasodilatory PGE and the degree of bleeding (Smith et al, 1981a; Baird et al, 1981), though an association between $PGF_{2\alpha}$ and objectively measured blood loss has also been proposed (Smith et al, 1982; Rees et al, 1984a).

The present study has again demonstrated a relationship between PGE and blood loss, however, it has also shown an association between MBL and "total" PG, or $PGF_{2\alpha}$, dependent on the criteria used to define the menorrhagic population.

Numerous reasons could account for the apparent inconsistency in cited data. Firstly, the measurement of the PGs themselves is fraught with difficulty. The compounds are produced readily when tissue is subjected to trauma, and so the very process of obtaining an endometrial biopsy will stimulate PG production (Green, 1979). It follows that care must be taken in interpreting studies, for the spurious elevation of PGs resulting from trauma during tissue collection does not necessarily reflect the in-vivo situation. Specimens obtained by biopsy and subjected to homogenisation

probably reveal the potential of the tissue to produce PGs on maximal stimulation. Such a measurement is of relevance in the assessment of PGs and menstruation, for it is likely that the influence of the PGs is actually exerted at the onset and during menstruation itself, following activation of the arachidonic acid cascade by the trauma of ischaemia and necrosis.

Alternatively the PGs have been measured using incubation systems (eg. with radio-labelled arachidonic acid) or superfusion/perifusion techniques. The use of the former method to obtain quantitative data has been questioned, as the variable arachidonic acid content of different tissues can have a marked effect on labelled-substrate metabolism (Dimov et al, 1983). Superfusion/perifusion techniques have been advocated in that a steady state is reached, presumably after the initial trauma-induced PG production has subsided (Liggins et al, 1980; Peek et al, 1985). However, it is mandatory to ensure tissue viability, for it may be that the steady state merely reflects the release of PGs from slowly dying tissues in the in-vitro situation. The method of PG assessment will affect the observed relationships with MBL, and Rees et al (1984b) using a superfusion technique, have failed to show any association between endometrial PGs and measured blood loss.

Comparison between studies must also take account of the actual method of PG measurement employed. The primary PGs are labile and are rapidly metabolised to more stable compounds. Metabolites such as 13,14 dihydro 15keto $PGF_{2\alpha}$ ($PGFM_2$) and 13,14 dihydro 15keto PGE_2 ($PGEM_2$) can be measured (Demers et al, 1983), and although not performed in the present work, this can provide additional information on specific prostanoid activity. PG metabolism can

also occur during assay, extraction, and sample storage, and attempts to overcome this have led to the use of bicyclo compounds (Granstrom et al, 1982) or methyl oxime derivatives, as in the present study (Kelly & Abel, 1983; Kelly et al, 1986a), prior to assay.

In addition, the wide variation in "normal" PG concentrations, the in-built variation (inter and intra) in the assay systems themselves, and other variables such as the true representative nature of the specimens obtained or the accuracy of the extraction procedures will all contribute to differences in reported data.

Finally, the precise relationship between the PGs and different study populations (i.e. women "with" and "without" menorrhagia) will depend upon the actual criteria used to define the study population itself.

Although the term "total" PG here has not included other arachidonic acid metabolites such as PGD_2 or TXA_2, the PGs E, $F_{2\alpha}$ and $6oxoF_{1\alpha}$ are thought to be the major prostanoid products of the endometrium. The suggested association between total endometrial PG and menstrual loss would account for the improvement in blood loss following the indiscriminate inhibition of both vasodilatory and vasoconstrictory PGs after cyclooxygenase blockade (Anderson et al, 1976). It would agree with the finding of increased $PGF_{2\alpha}$ and PGE_2 in the menstrual fluid of menorrhagic women (Rees et al, 1984a). In addition, Kelly et al (1984) have reported an increased availability of endometrial precursor arachidonic acid (and therefore presumably a potential increase in PGs) in women with heavy periods.

The synthesis of endometrial PGs is represented in figure 4.3 (page 78). An increased availability of arachidonic acid would result in an increased availability of the cyclic endoperoxides PGG_2 and PGH_2. These in turn will lead to an increased synthesis of the primary PGs, mainly PGE_2 and $PGF_{2\alpha}$. As the conversion to PGE_2 can occur both via the isomerase and non-enzymatically, one consequence of an increased precursor endoperoxide load could be an increase in the PGE_2:$PGF_{2\alpha}$ ratio (Smith et al, 1981a and 1982), which would depend on the saturation level of the PGF reductase enzyme.

The Effects of medical treatment for menorrhagia

Irrespective of the small number of cases, no consistent effect was seen on endometrial PG concentrations after treatment with danazol, despite a marked improvement in the degree of blood loss. Major actions of danazol include binding to and activation of androgen receptors, displacement of testosterone from sex hormone binding globulin, and a decrease in the hepatic synthesis of this protein. These actions increase testosterone concentrations and subsequently stimulate androgen receptor-mediated effects in the endometrium (Dmowski, 1979; Tseng et al, 1982; Bevan et al, 1984). The improved blood loss may therefore be related to a reduction in endometrial mass, which would be supported by the inability to obtain endometrial biopsies in 2 of the 6 women under investigation here.

Mefenamic acid, on the other hand, is known to act mainly as a cyclooxygenase inhibitor. Nevertheless, no effect was seen on

endometrial PG concentrations in the present study. However, it should be noted that the drug was taken for the first 5 days of menses, and the biopsies were obtained in the mid-luteal phase some 2½-3 weeks later - it is perhaps not surprising, therefore, that no inhibition of the arachidonic acid cascade was seen. Previously cited data on the endometrial effects of orally administered PG synthetase inhibitors are conflicting. Shapiro & Haning (1983) observed no effect on endometrial $PGF_{2\alpha}$ concentrations during the mid luteal phase after ibuprofen ingestion, but showed a significant decrease in the PG when biopsies were taken within the first few hours of menstruation. This could be related to their much lower concentrations of $PGF_{2\alpha}$ in premenstrual control endometrium (0.3 pg/mg) compared with that during menstruation (7.0 pg/mg). The clinical efficacy of the drug itself was not assessed.

Using mefenamic acid, Fraser (1983) investigated nine women with menorrhagia, in 4 of whom treatment had been unsuccessful. In endometrial biopsies taken within 4 hours of drug administration, PGE_2 and $PGF_{2\alpha}$ concentrations were suppressed in the 5 women who were classified as treatment successes, but the 4 "treatment failures" did not exhibit significant prostanoid inhibition. Again though, this group of women had lower pre-treatment concentrations of endometrial PGs than the treatment successes. Further studies are required to clarify this situation.

The use of the progesterone-impregnated coil in the present study resulted in both a significant improvement in the degree of MBL and a decrease in the endometrial concentrations of PGE, $PGF_{2\alpha}$ and "total" PG. There are varied reports on the effects of intrauterine devices on endometrial PG production. Saksena &

Harper (1974) demonstrated an increase in F prostaglandins in the cornu of rabbit uteri bearing an intrauterine device, but in women, the main increase was of PGE (Hillier & Kasonde, 1976). However others (Green & Hagenfeldt, 1975; Scommegna et al, 1978) have failed to show a consistent change in endometrial prostanoids after coil insertion. The variance in results is probably related to methodological differences in PG extraction and measurement, the difficulty in obtaining a representative endometrial sample in close proximity to the coil itself, and the varied duration of intrauterine device usage prior to biopsy. Employing the same medicated coil as in the present study though, both Troughbough et al (1978) and Zahradnik et al (1978) noted a reduction in endometrial $PGF_{2\alpha}$. It may be that the continuous administration of progesterone throughout the cycle results in an impairment of estradiol receptor generation (Shaw et al, 1981; West & Brenner, 1985), which in turn could lead to impaired estradiol-mediated cyclooxygenase activation, and a decrease in prostaglandin synthetic capacity. Alternatively, the progesterone may stimulate PG metabolism (Casey et al, 1980; Abel & Kelly, 1983), thereby reducing the observed tissue concentrations. Further studies assessing endometrial steroid receptors or PG metabolite concentrations are therefore required to test these hypotheses.

Finally, with norethisterone there were no changes in either MBL or endometrial PG concentrations. It would be pertinent though to observe the effect of this synthetic gestogen on endometrial PG production, when administered in the second half of the cycle to women with anovulatory dysfunctional bleeding.

CHAPTER 4

ENDOMETRIAL-MYOMETRIAL INTERACTION

The pattern of prostaglandin production from isolated endometrial biopsies has been outlined in Chapter 3, however in vivo the endometrium bears a close relationship, both anatomically and functionally, with the large muscle mass of the myometrium. It is therefore apposite to examine this relationship in vitro.

In contrast to the endometrium, 6oxo$PGF_{1\alpha}$ is the major myometrial prostanoid in the rat (Fenwick et al, 1977), sheep (Jones et al, 1977) and man (Abel & Kelly, 1979). Whether prostaglandins of the E and F series are also synthesised in the myometrium is less certain. Although a cyclical variation in both PGE_2 and $PGF_{2\alpha}$ has been suggested (Vijayakamur, 1980), with a peak myometrial concentration of both PGs at the time of ovulation, and a secondary rise in PGE_2 content during menstruation, others have failed to show a significant production of these PGs in uterine muscle (Abel & Kelly, 1979).

Using broken cell preparations, both with and without incubation with ^{14}C-labelled arachidonic acid, an interaction between the endometrium and myometrium has been demonstrated (Abel & Kelly, 1979; Kelly et al, 1984), with an increased production of 6oxo$PGF_{1\alpha}$ when the two uterine tissues are combined. Furthermore, Smith et al (1981b) reported that myometrial prostacyclin production could be enhanced on co-incubation of the muscle with endometrium from women with objectively diagnosed menorrhagia.

The present work has further investigated the interaction between homologous endometrium and myometrium, using intact pieces of endometrium.

METHODS

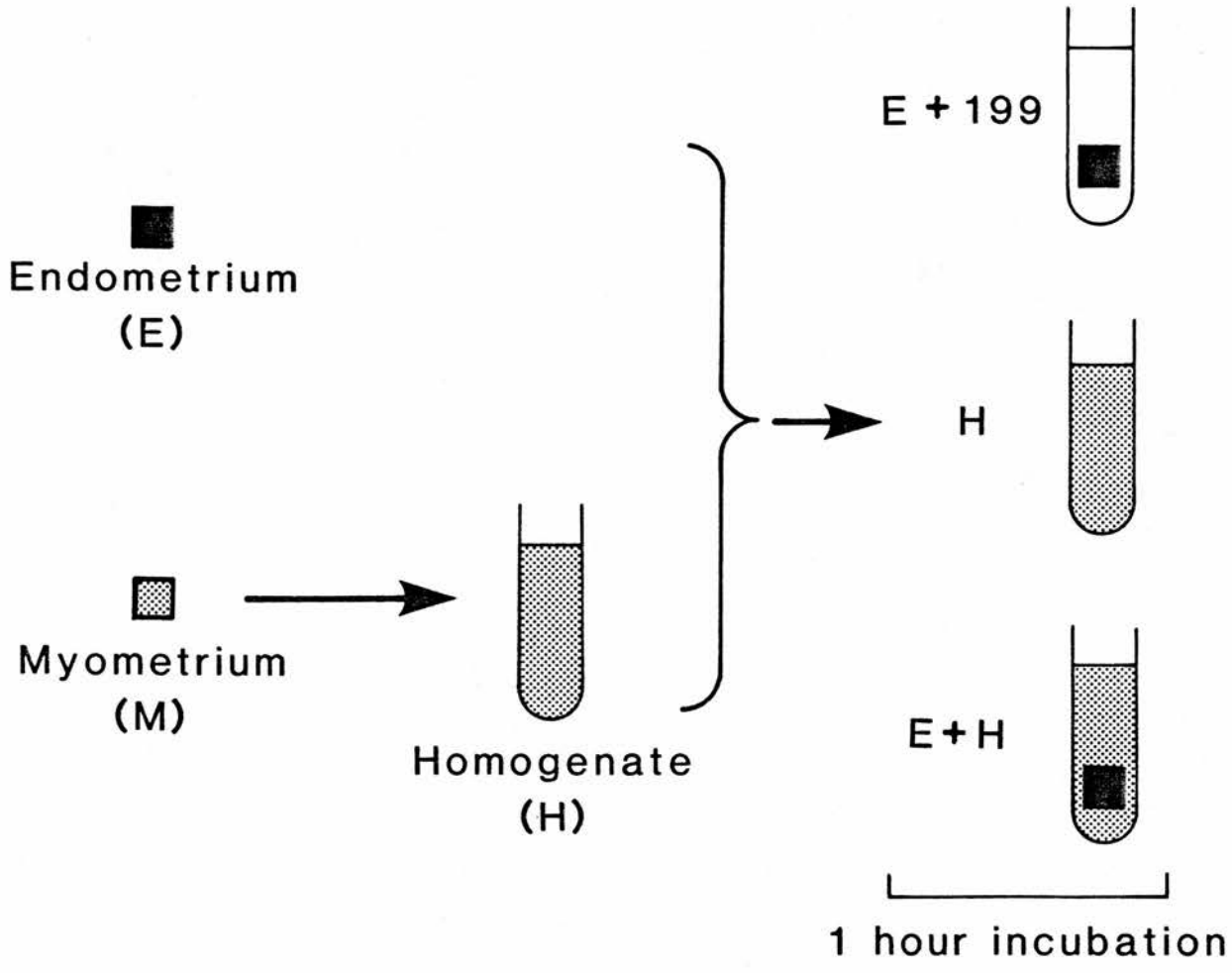
Tissue Collection and Prostaglandin Measurement

6 women suffering from benign uterine disease, each with a subjective complaint of menorrhagia were admitted for abdominal hysterectomy in the luteal phase of the cycle. At operation, samples of both endometrium and myometrium were placed in ice-cold modified 199 medium and transported to the laboratory. After blotting and weighing both types of tissue, a myometrial homogenate was prepared in 199 medium (using a Polytron homogeniser), at a final concentration of 10 mg/ml. Thereafter, small pieces of endometrium (4.3 mg (1.9, 10.5) - median (range) - n = 48) were placed in either 1 ml of 199 medium or 1 ml of the myometrial homogenate, and these samples were incubated at 37^{o}C in a water bath for 1 hour, with gentle shaking (figure 4.1). 1 ml samples of myometrial homogenate alone, and control samples of 199 medium were also processed in similar fashion. Following the incubation, the pieces of endometrium were removed, and the media were stored at -20^{o}C until assayed.

Prostaglandin concentrations were measured by radioimmunoassay as described in Chapter 3.

Endometrial Dating

In all experiments, a portion of endometrium was placed in formol saline for histological dating. The occurrence of ovulation was indicated by the presence of secretory endometrium, along with a serum progesterone concentration greater than 18 nmol/L.


E + 199
Endometrium
(E)
H
Myometrium
(M)
Homogenate
(H)
E+H
1 hour incubation

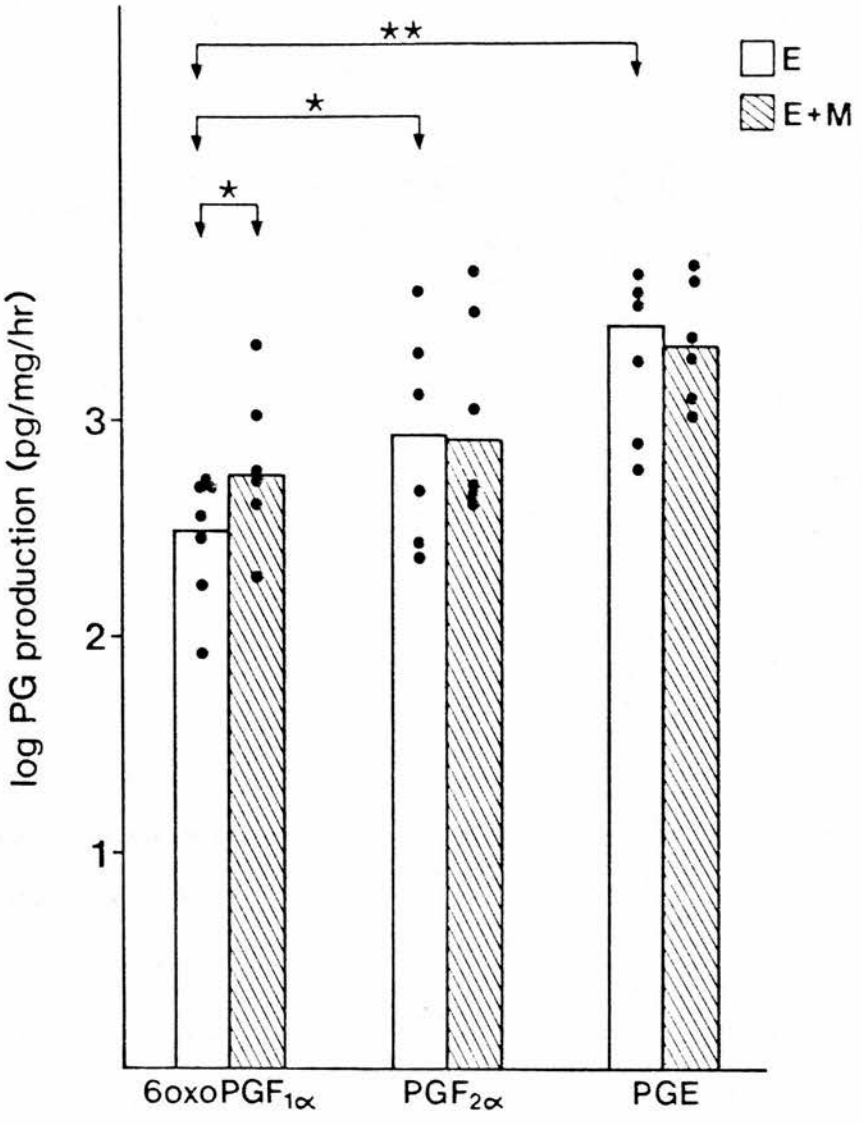
RESULTS

The characteristics of the 6 patients are seen in table 4. All were in the secretory phase of the cycle, and all showed biochemical and histological evidence of luteal function. Each of the women complained of heavy periods, with associated dysmenorrhoea in two cases, however their menstrual loss was not assessed objectively.

	AGE(yrs)	PARITY	CYCLE	DAY
1	46	3+0	4/28	22
2	43	1+0	8/25	26
3	46	4+2	8/26	25
4	35	4+1	11/24	24
5	40	2+0	7/23	22
6	40	4+0	6/21	17

Table 4. Patient characteristics

Endometrial prostaglandin release, both alone and in combination with myometrium, is presented in figure 4.2. The value for the combined incubate has been expressed in pg/mg endometrium per hour, after correcting for the amount of PG derived from the myometrial homogenate. Alone, the endometrial production of 6oxoPGF$_{1\alpha}$ was significantly less than that of either PGF$_{2\alpha}$ or PGE$_2$. There was an increase in the production of 6oxoPGF$_{1\alpha}$ by the endometrium on co-incubation, however, there was no significant change in the production of PGF$_{2\alpha}$ or PGE.


**
*
*
E
E+M
log PG production (pg/mg/hr)
3
2
1
6oxoPGF1α
PGF2α
PGE

The median (range) production of 6oxoPGF$_{1\alpha}$, PGF$_{2\alpha}$ and PGE from the myometrial homogenate alone was 186 pg/mg/hr (54,1109), 87 pg/mg/hr (36,101) and 70 pg/mg/hr (10,239). There was no statistical difference between the production of these 3 prostanoids.

DISCUSSION

The present work further supports the hypothesis that when endometrial and myometrial tissues are combined, the endometrium can supply precursor endoperoxide to the myometrium, where it is converted primarily to prostacyclin and thence to 6oxoPGF$_{1\alpha}$. That it has not been necessary to prepare an endometrial homogenate to demonstrate this increase in 6oxoPGF$_{1\alpha}$ production would suggest that the intact endometrium could release precursor endoperoxide in-vitro. Passage of endoperoxide from the myometrium to the endometrium would seem unlikely in the absence of an increase in PGE and PGF$_{2\alpha}$ on co-incubation.

A corresponding reduction in PGE and PGF$_{2\alpha}$ production was not seen on combined incubation, in contrast with previous data (Abel & Kelly, 1979). This finding could be accounted for by differences in experimental design, however a decrease in the production of the PGs E and F$_{2\alpha}$ resulting from the diversion of endoperoxide towards prostacyclin synthesis would only be expected in the face of a limited supply of precursor endoperoxide itself.

Production of both PGE and PGF$_{2\alpha}$ was detected in the myometrial homogenate alone. The capacity of the myometrium to synthesise these PGs is debated (Abel & Kelly, 1979; Vijayakamur, 1980),

however, the risk of endometrial contamination must be considered in the interpretation of such data.

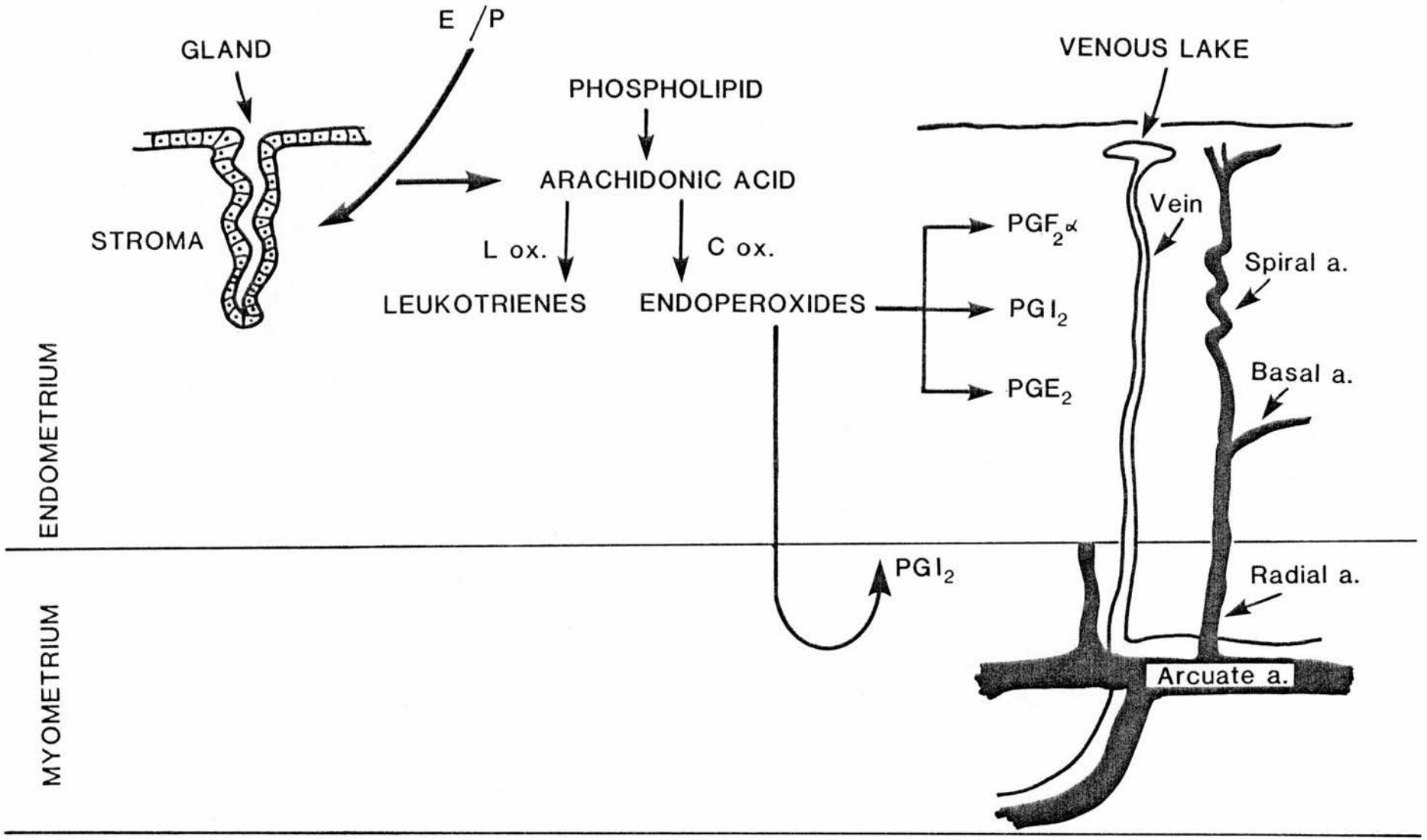
The variable degree by which the 6 oxo $PGF_{1\alpha}$ concentration increased in the present experiments (by a factor of 1.3 to 6.7 times), might have been related to the level of menstrual blood loss. However, in view of the inadequacies of the subjective assessment of MBL (Chapter 2), future studies recruiting women with objectively assessed periods would be necessary to test this further.

In conclusion, the presented work illustrates that an interaction between endometrial and myometrial prostaglandin production is possible, and that precursor endoperoxide can be released from intact endometrium. Although such <u>in vitro</u> data do not necessarily prove that this mechanism exists <u>in vivo</u>, the diversion of precursor endoperoxide towards myometrial prostacyclin production could have important implications in the pathogenesis of menorrhagia.

PROSTAGLANDINS and MENSTRUATION: CONCLUSION

How can the data from the foregoing chapters be summarised in an overall plan explaining the role of the PGs in the mechanism of menstruation, and what is its clinical significance?

The proposed interrelationship between PGs and the uterine vasculature is schematically represented in figure 4.3. It is likely that the PGs regulate the degree of blood loss, and that in vivo this occurs at the time of menstruation itself. The potential for PG production at menstruation is dependent on the actions of estradiol and progesterone to induce cyclooxygenase activity and facilitate arachidonic acid storage respectively. Whether the PGs are directly involved in the physiological initiation of menstruation is unclear, though bleeding can be induced by these compounds (Wiqvist, 1971), and $PGF_{2\alpha}$ could be the vasoconstrictive agent seen by Markee (1942) at the onset of menstruation in his experimental model. However, once the process has begun, the resultant ischaemic necrosis of the endometrium will lead to activation of the arachidonic acid cascade. The presented data suggest that the degree of menstrual blood loss may be related to the subsequent production of PGs. The observations supporting a relationship between total PG and the amount of bleeding have been summarised in Chapter 3, and an increase in prostanoid precursor load will result in not only an increase in total PG and an increase in the PGE : $PGF_{2\alpha}$ ratio, but also in an increased production of PGI_2, both in the endometrium, and in the large muscle mass of the myometrium (Chapter 4). Although synthesised in small amounts per gramme of tissue compared with the other primary PGs, the overall myometrial production of prostacyclin will be relatively large, and with its inherent powerful pharmacological


GLAND
E /P
PHOSPHOLIPID
ARACHIDONIC ACID
STROMA
L ox.
C ox.
LEUKOTRIENES
ENDOPEROXIDES
PGF2α
PGI2
PGE2
VENOUS LAKE
Vein
Spiral a.
Basal a.
Radial a.
Arcuate a.
PGI2
ENDOMETRIUM
MYOMETRIUM

activity, this compound could contribute significantly to the degree of blood loss.

The prostaglandins though are not the only agents that may play a part in the local control of menstrual bleeding. In particular it has been suggested that there may be an increased activation of the fibrinolytic system in the endometrium of women with heavy periods (Bonnar et al, 1983), and such an abnormality is upheld by the successful use of fibrinolytic inhibitors to improve MBL (Ylikorkala & Viinikka, 1983). In addition, interactions may occur between both the PGs and the fibrinolytic system, and other endometrial products such as prolactin (Healy & Cameron, 1987).

A better understanding of the mechanism underlying menorrhagia should lead to a more discerning approach to the clinical management of abnormal menses. The significant morbidity attributed to disorders of menstruation has already been mentioned (Chapter 1), and in addition the pitfalls in the subjective diagnosis of these disorders have been demonstrated (Chapter 2). Surely there is now sufficient data to indicate the necessity for objective assessment prior to subjecting women to major surgery as a treatment option?

Improved knowledge of the pathogenesis of menorrhagia should also lead to advances in the availability of medical treatments for heavy menses. Alternative approaches to PG inhibition could be via phospholipase inactivation (Mitchell et al, 1977). Furthermore it is likely that systemic side effects could be reduced by considering a variety of local therapies, such as medicated

intra-uterine devices incorporating prostaglandin or fibrinolytic inhibitors (Tauber et al, 1981).

Matthews Duncan said, "Menstruation is like the red flag outside an auction sale. It shows that something is going on inside." (Beckwith Whitehouse, 1914). That something is going on is without question, and it would appear that the prostaglandins play a major part.

CHAPTER 5

THE THERAPEUTIC APPROACH TO MENSTRUAL INDUCTION

The role of the prostaglandins in the mechanism of menstruation has been discussed. Endometrial changes leading to menstrual bleeding are subsequent to alterations in the pattern of ovarian steroid production, and the resultant degree of blood loss appears to be controlled, at least in part, by specific endometrial prostanoids.

However, besides being implicated in the physiological processes underlying normal menstruation, the isolation of the natural prostaglandins, and the development of numerous synthetic analogues, has led to the use of the prostanoids as therapeutic agents. One clinical application has been the introduction of the prostaglandins as a medical means of inducing menstruation, and with the increasing demand for the use of very early abortion as a method of fertility control, this subject has received much recent attention.

The second part of this thesis will assess the therapeutic approach to menstrual induction using one synthetic prostaglandin analogue (16,16,dimethyl-trans-Δ_2-PGE_1 methyl ester: Smith & Baird, 1980) both alone, and in combination with the progesterone receptor antagonist RU486 (Baulieu, 1985b). These treatments will be compared with conventional surgical techniques, and consideration will also be given to the endocrine effects of early pregnancy interruption on subsequent ovarian function.

Firstly though, it is appropriate to outline the potential clinical impact of, and the theoretical approach to, menstrual induction.

Since the introduction of the 1967 Abortion Act, pregnancy termination has become a significant method of fertility control in

the United Kingdom (Office of Population Censuses and Surveys, 1985). Over 80% of cases present in the first trimester and surgical evacuation of the uterus remains the most popular treatment method.

Surgical techniques

Vacuum aspiration was first reported by Simpson in 1863, and at present flexible plastic cannulae are used most often (Karman & Potts, 1972). The main side effects associated with this treatment appear to be related to trauma, to the anaesthetic methods used, and to bleeding and infection following operation.

The cervix is especially susceptible to trauma during dilatation (Johnstone et al, 1976), and the necessity for generous dilatation is related to the gestational age. If less than 56 days have elapsed since the last menstrual period, minimal dilatation is required, and this may be achieved by the insertion of progressively larger plastic cannulae until the appropriately sized instrument is reached. Trauma to the body of the uterus is uncommon, especially in early pregnancy, and in over 10,000 reported cases of menstrual regulation the incidence of uterine perforation has been documented as less than 0.03% (Edelman and Berger, 1981).

A variety of anaesthetic methods are available for surgical abortion, and although many centres continue to use general anaesthesia, local anaesthetic techniques have become increasingly popular (Meyer, 1983). Methods of vacuum aspiration, as opposed to dilatation and curettage, besides providing a more complete uterine

evacuation with less blood loss, have made such local anaesthetic techniques more feasible, and in very early pregnancies, particularly in parous women, anaesthesia itself may not be necessary (Stringer et al, 1975).

Local anaesthetics are usually administered by paracervical block. Although this avoids the theoretical complications associated with general anaesthesia, the overall complication rate appears to be similar with the two techniques (Soderstrom, 1979).

Bleeding usually occurs for about 7-10 days after suction evacuation, and a similar bleeding pattern has been observed in prostaglandin - induced abortion, both in terms of the duration of blood loss, and its measured volume (Smith & Baird, 1980).

The infection rate in early surgically-terminated pregnancies has been estimated as 1.7% (Edelman & Berger, 1981). However, stringent follow-up arrangements are necessary (though this is often difficult in women who request abortions) in order to obtain a realistic figure. Infection may be related to the completeness of uterine evacuation, and the incidence of both retained products and failed abortion is greater in early pregnancy (<42 days amenorrhoea) than it is later in the first trimester (Hern, 1984; Nesheim, 1984).

Medical methods of pregnancy interruption

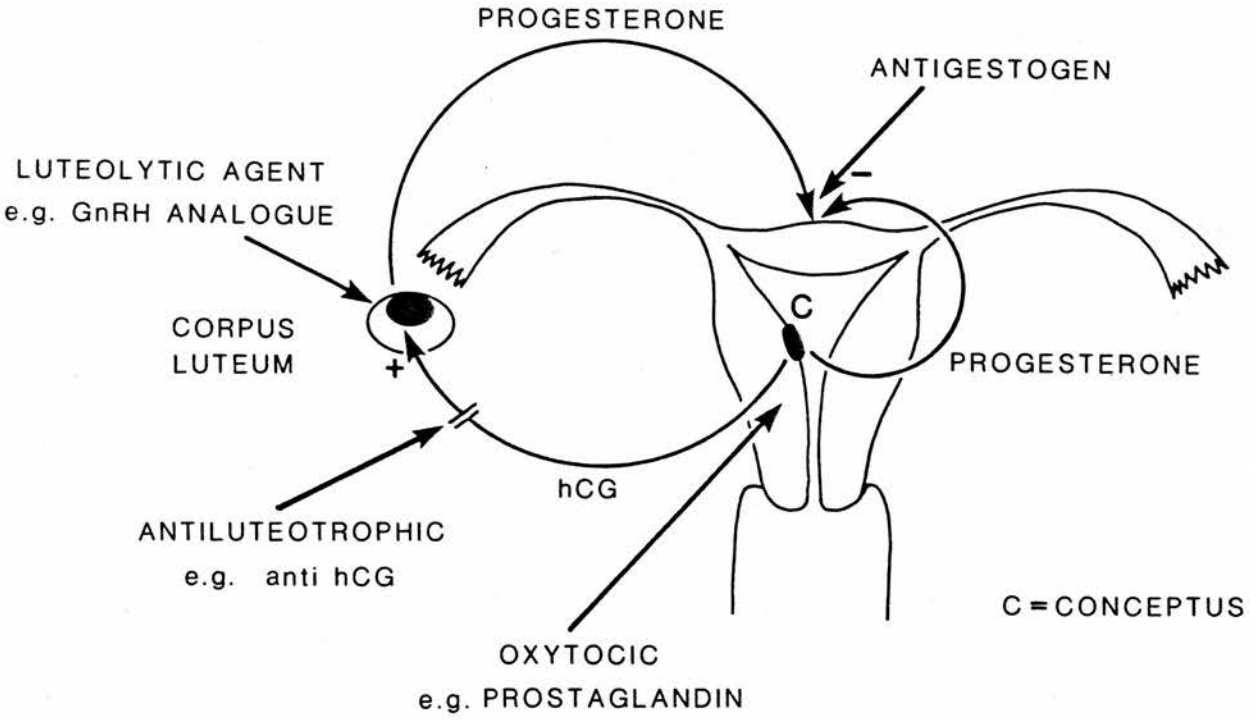
An awareness of these adverse effects, coupled with an increased demand for the use of early abortion as a routine method of

fertility regulation, has led to the assessment of medical agents for menstrual induction.

There are additional advantages to the development of reliable medical methods for post-conceptional fertility control. The treatment need only be used when required, obviating the need for the chronic ingestion of synthetic steroids as in the oral contraceptive pill. Furthermore, medical agents for menstrual induction offer a favourable alternative to surgery for women themselves (Rosen et al, 1979). Such treatment also lends itself to application on a wide scale in developing countries with considerable population problems, but without the resources to provide trained surgical personnel (Purandare et al, 1982; Aegostina et al, 1984).

The various therapeutic approaches to menstrual induction are shown in figure 5. C represents the growing conceptus, secreting chorionic gonadotrophin, which acts to maintain the corpus luteum. Both the corpus luteum and the trophoblast produce steroids including progesterone, and this hormone has an essential role in the development of the embryo and in maintaining uterine quiescence (Csapo, 1973b).

A variety of agents can be employed to interrupt this equilibrium. The use of luteolytic compounds such as the GnRH analogues has had limited application because their action can be overcome by endogenous hCG (Fraser, 1981). Although uterine $PGF_{2\alpha}$ is the naturally-occurring luteolytic agent in many species, such as the guinea pig, rat and sheep (Horton & Poyser, 1976), this does not


PROGESTERONE
ANTIGESTOGEN
LUTEOLYTIC AGENT
e.g. GnRH ANALOGUE
CORPUS
LUTEUM
+
C
PROGESTERONE
hCG
ANTILUTEOTROPHIC
e.g. anti hCG
C = CONCEPTUS
OXYTOCIC
e.g. PROSTAGLANDIN

appear to be the case in man (Baird, 1985). However, the prostaglandins have shown promise as menstrual inducers, related to both their oxytoxic properties (Karim & Filshie, 1970) and their progesterone - withdrawal effects (Csapo & Pulkkinen, 1979 a & b).

The Prostaglandins

Initially the naturally-occurring prostaglandins were used (Karim, 1971), however, their widespread actions, especially following systemic administration, resulted in frequent side effects. An additional problem with the natural compounds was their inherent lability, and a number of synthetic analogues have now been produced with increased stability and more specific uterine activity (Oshima et al, 1978). Analogues of both the E and F series have been synthesised (MacKenzie et al, 1978; Bygdeman et al, 1980; Smith and Baird, 1980; Csapo et al 1982), and these have often been administered in the form of vaginal pessaries. Although the success rate for abortion induction is high (85-95%), troublesome gastro-intestinal associated side effects are still seen in up to 40% of cases despite local methods of drug administration (Bygdeman & Green, 1979; Bygdeman et al, 1980; Smith and Baird, 1980). However, care should be taken when comparing such studies, not only because of the differing uterine specificity of various analogues, but also because of inconsistencies in the use of routine prophylactic premedication (Bygdeman et al, 1980; Foster et al, 1985).

There has been debate regarding the precise mechanism of action of the PGs in the induction of early abortion. Further evidence has

accumulated opposing a luteolytic role; Takagi et al (1978) failed to show a fall in the plasma concentration of 17 hydroxyprogesterone after the administration of 16,16 dimethyl-trans- Δ_2-PGE_1 methyl ester pessaries, and this finding has been confirmed (Tan et al, 1984). These workers noted a reduction in plasma estradiol and progesterone concentrations following therapeutic abortion with 16,16 dimethyl PGE_2 p-benzaldehyde semicarbazone ester, and postulated a direct effect of the prostaglandin on trophoblast steroidogenesis.

That the major role of the prostaglandins is not entirely via myometrial stimulation was reported in a series of experiments by Csapo and colleagues (Csapo et al, 1976; Csapo & Pulkkinen, 1979 a & b), who demonstrated that changes in intrauterine pressure and cervical dilatation did not occur in unison with the administration of exogenous prostaglandin. They suggested that the mechanism of prostaglandin action, the "prostaglandin impact", resulted in progesterone withdrawal. This would convert the pregnant uterus from a quiescent to an activated state, following which abortion could proceed mediated by endogenous (or supplementary exogenous) prostanoids.

As a means of interrupting early pregnancy, progesterone withdrawal is highly effective. At early gestations the major progesterone source is the corpus luteum, and the importance of the corpus luteum in the maintenance of pregancy was elegantly demonstrated in luteectomised rabbits using porcine corpus luteum extracts (Allan & Corner, 1930). Subsequently Csapo and co-workers confirmed these findings in women, and established that the effects of luteectomy

on early pregnancy could be reversed by exogenous progesterone (Csapo et al, 1973b).

The supportive role of progesterone in early pregnancy has been further demonstrated with the development of relatively specific "anti-progesterone agents". Progesterone withdrawal using epostane (a competitive inhibitor of the 3ß hydroxy steroid dehydrogenase enzyme system) appears to sensitise the myometrium to exogenous oxytocics in early pregnancy (Webster et al, 1985a), and at high doses it is an effective menstrual inducer itself (Webster et al, 1985b). Alternatively progesterone receptor antagonisation using RU 486 (17ß-hydroxy-11ß-(4-dimethylaminophenyl)-17 (prop-1-ynyl)-estra-4,9-dien-3-one) offers the potential for inducing early abortion with a low incidence of side effects (Baulieu, 1985a). However, it has been suggested that although the antigestogens are effective at interrupting pregnancy itself, they may not induce sufficient uterine activity to expel the products of conception, and recently an abortion rate approaching 100% has been demonstrated using RU486 treatment followed by one intramuscular injection of 16-phenoxy-tetranor-PGE_2 methyl sulfonlyamide (Swahn et al, 1985a).

The following chapters discuss the use of both 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester (Chapter 6) and RU486 (Chapter 7) for the induction of menstruation in early pregnancy. In addition the effects of menstrual induction on subsequent ovarian function will be examined (Chapter 8).

CHAPTER 6

MENSTRUAL INDUCTION USING 16,16,dimethyl-trans-Δ_2-PGE_1 methyl ester : A COMPARISON WITH VACUUM ASPIRATION

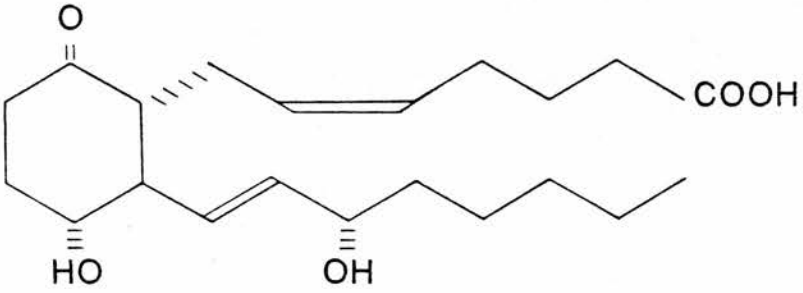
As outlined in Chapter 5, until recently the prostaglandins alone offered the most promising therapeutic approach to menstrual induction. However their inherent lability and high incidence of side effects has limited their therapeutic acceptability.

Although a lower dose of prostaglandin can be used to decrease the incidence and severity of these side effects, this may lead to a reduced success rate for abortion itself. One solution would be to administer the prostaglandin in a controlled-release form, thereby lessening the "bolus effect" each time that an individual dose of drug is given. The feasibility of such slow-release systems has been demonstrated in primate models (Spilman et al, 1984), and clinical studies have confirmed the efficacy of various devices both for inducing labour (Embrey et al, 1980), and abortion (Bygdeman et al, 1984).

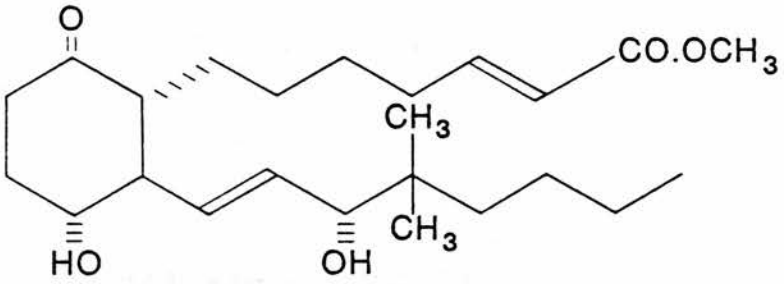
The present study has investigated the use of one synthetic prostaglandin analogue, 16,16 dimethyl-trans- Δ_2-PGE_1 methyl ester (figure 6.1) which has been used extensively for the interruption of pregnancy in both the first (Takagi et al, 1978; Smith and Baird, 1980) and second trimesters (Cameron and Baird, 1984). The analogue has been used here both in pessary form (1mg prostaglandin in 0.8 gm. of Witepsol S-52 base), and incorporated into a controlled-release system (NcNeill and Graham, 1984). Both methods of treatment have been compared with conventional vacuum aspiration under general anaethesia.

PATIENTS AND METHODS

73 women with pregnancies of 56 days amenorrhoea or less were



PGE_2



16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester

recruited for study. Gestational age was assessed by menstrual history and clinical examination, with the addition of pelvic ultrasound where necessary. In all cases, pregnancy was confirmed with an immunological pregnancy test (Hobson, 1968). Patients with evidence of abnormal pregnancy or spontaneous abortion were excluded from study, as were women with medical complications such as cardiovascular or pulmonary disease, allergy, or epilepsy. Pregnancy termination was indicated under the 1967 Abortion Act: local ethical committee approval was granted for the project, and written informed consent was obtained from each woman prior to proceeding with treatment.

Once recruited, patients were allocated to the appropriate study group. The first 60 women were randomised to receive either 16,16 dimethyl-trans- Δ_{2}-PGE_1 methyl ester in pessary form, or vacuum aspiration under general anaesthesia. The remaining 13 individuals were treated with the prostaglandin analogue administered in a controlled-release device.

Vacuum aspiration

Treatment was performed on an outpatient basis. The cervix was dilated to a maximum of 8mm, and the uterine cavity was evacuated using a size 6 or 8 Karman cannula (Karman and Potts, 1972). Pre-operative cervical priming was not used, but all women received either I0 U oxytocin (Syntocinon: Sandoz Products, Ltd) or 500µg ergometrine maleate and 5U oxytocin (Syntometrine: Sandoz Products, Ltd) immediately prior to, or during the procedure itself.

16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester: Pessaries

A single 1mg pessary was placed in the posterior fornix of the vagina, and this treatment was repeated every 3 hours until either the products of conception had been expelled, the frequency or severity of any side effects became so great as to warrant cessation of therapy, or until a total of 5 pessaries had been given.

16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester: Controlled-release device

The device, containing 3mg of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester incorporated into a strip of polyethylene-oxide based hydrogel (measuring 1.3x10x3mm) was placed in the posterior fornix of the vagina. It was removed prior to discharge from hospital the following morning.

After one hour women in both the prostaglandin groups were allowed to get up, and they remained in hospital for 24 hours. Prophylactic medication was not prescribed, however analgesics (paracetamol, 1gm; dihydrocodeine 30 mg; or pethidine 100mg), antiemetics (cyclizine, 50mg) or other preparations such as antidiarrhoeal agents, were administered on request. No dietary restriction was enforced during treatment.

All patients were given appropriate contraceptive advice before going home, and arrangements were made for follow-up at 1 week, 2 weeks, 4 weeks and fortnightly thereafter, until the onset of the

next period. Abortion was considered successful at the 2 week visit if there was minimal vaginal bleeding, the cervix closed, the uterus was normal in size, and the pregnancy test indicated that the concentration of human chorionic gonadotrophin was falling. In the event of treatment failure, the uterus was evacuated under general anaesthesia.

RESULTS

Patient characteristics

Two women in the vacuum aspiration group were excluded from analysis, having been found to have pregnancies of greater than 56 days amenorrhoea. The characteristics of the remaining women are shown in table 6.1. There were no major differences between the treatment groups.

	V.A.(28)	PG:PESSARY(30)	PG: DEVICE(13)
AGE (years)	23,(19,39)	22(18,38)	21(16,45)
HEIGHT (cm)	168(160,180)	168(154,179)	165(155,168)
WEIGHT (kg)	59(52,67)	55(57,79)	58(56,73)
PARITY >28W	5(18%)	5(17%)	4(31%)
<28W	5(18%)*	4(13%)	1(8%)*
GESTATION (days)	49(34,56)	49(33,56)	52(43,56)

Table 6.1 Patient characteristics. The parity, both greater than and less than 28 weeks is expressed as the number of parous women with the percentage in parentheses. Other figures represent the median (range). (V.A. - vacuum aspiration; PG: pessary - prostaglandin analogue in pessary form; PG: device - prostaglandin analogue in slow release form).*p= 0.02.

Vacuum aspiration

All 28 women received outpatient treatment as planned, without complication. Blood loss was not measured objectively, but in no case was transfusion or overnight observation required.

Prostaglandin

28(93%) women received the maximum 5x1mg pessaries. The other 2 individuals in the pessary group aborted gestational sacs after receiving the 4th pessary, and treatment was therefore discontinued.

All patients experienced crampy period-like pain of varying severity. The pain began after 240 minutes (median) in both the pessary and device group, and this was followed by the onset of bleeding after 507 minutes (range 267,1440) and 390 minutes (180,985) respectively in the 2 groups. The difference was not statistically significant.

Analgesic requirements were similar in the 2 groups, as presented in table 6.2.

	PG: PESSARY	PG: DEVICE
ANALGESIC		
ORAL	14(47%)	5(38%)
PARENTERAL	16(53%)	6(46%)
ALL	25(87%)	11(85%)

Table 6.2 Analgesic requirements. The number of individuals requiring pain relief is shown, with the percentage in parentheses. (Oral - paracetamol 1gm or dihydrocodeine 30mg; Parenteral - pethidine 100mg or diamorphine 5-10mg, both given intramuscularly with cyclizine 50mg).

During treatment 23(77%) women in the pessary group and 9(69%) individuals in the device group experienced a short lived pyrexia of 37-38^{o}C. The temperature settled spontaneously, and in no case was there clinical evidence of sepsis.

There were no serious side effects. Actual blood loss was not measured, but on no occasion was the subjective loss so great as to warrant either blood transfusion or emergency uterine evacuation. However, vomiting and diarrhoea occurred in 7(23%) and 10(33%) of the pessary group and 2(15%) and 3(23%) of the device group (N.S., $p>0.05$).

Follow up and success

Vaginal bleeding persisted for 7 days (range 1,25), 10 days (1,17) and 9 days(6,21) in the vacuum aspiration, pessary and device groups respectively. In these same groups crampy pain occurred during the first week in 6(32%), 13(43%) and 7(54%) women.

Successful interruption of pregnancy without the need for supplementary surgical intervention was seen in 27(96%) women in the vacuum aspiration group, 29(97%) in the pessary group, and in 11(85%) of the women receiving the controlled-release prostaglandin. The success rate did not differ statistically between the 3 groups. The fate of those women requiring uterine evacuation is shown in figure 6.2.

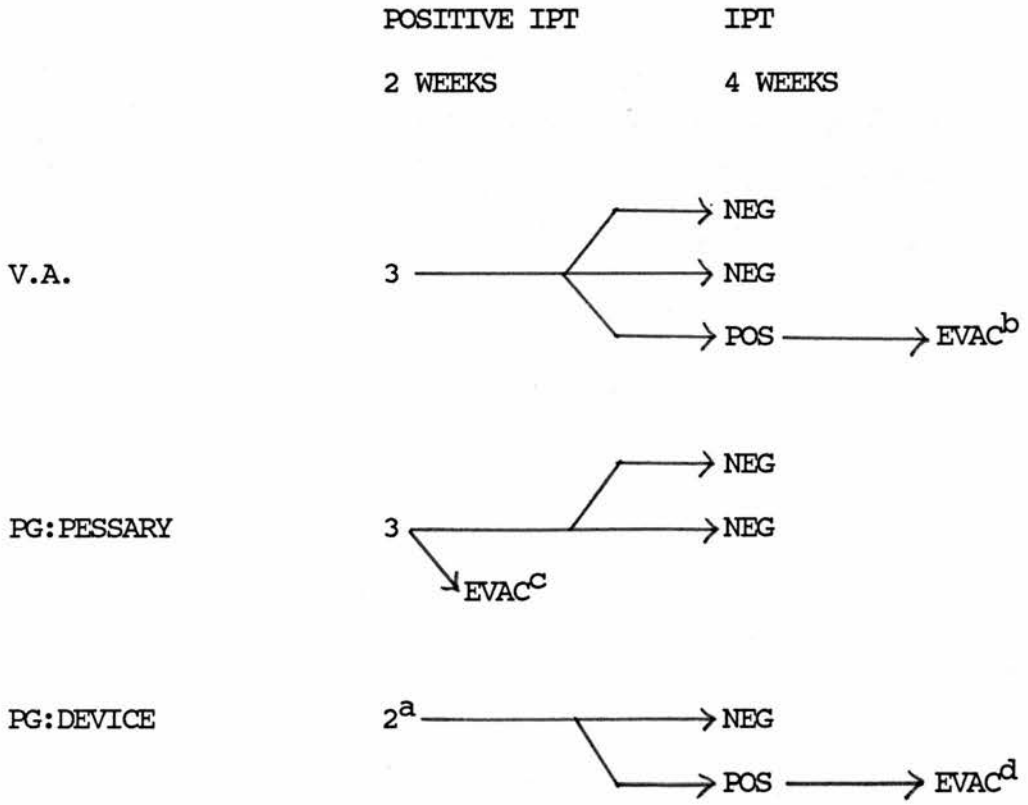


Figure 6.2

Treatment failures. The fate of each individual with a positive pregnancy test (IPT) 2 weeks following treatment is shown:

a - one patient had undergone uterine evacuation (EVAC) prior to the 2 week appointment.

b - curettage revealed necrotic decidua but no villi. The IPT was weakly positive.

c - uterine evacuation performed at 2 weeks because of ongoing pregnancy

d - curettage revealed chorionic villi.

(V.A. - vacuum aspiration; PG:pessary - prostaglandin analogue in pessary form; PG:device - prostaglandin analogue in slow-release form).

Table 6.3 details the haemoglobin concentration and white cell count, taken before treatment, and at the 4 week follow up appointment.

	Hb(g/dl)		WCC($x10^9$/l)	
	Pre	Post	Pre	Post
V.A.	12.7(11.5,13.9)	13.0(11.7,14.2)	8.1(4.7,12.5)	6.5(4.9,9.4)
PG: PESSARY	12.6(11.2,14.0)	12.6(11.0,14.1)	7.5(4.9,11.1)	7.1(4.7,12.3)
PG: DEVICE	12.9(11.1,14.2)	12.8(11.6,13.6)	8.9(4.7,14.7)	6.5(5.2,8.9)

Table 6.3 Haematological parameters. The haemoglobin (Hb) concentration and white cell count (WCC) are presented, before treatment (pre) and at the 4 week follow up (post), as the median (range).
(VA - vacuum aspiration; PG: pessary - prostaglandin analogue in pessary form; PG: device - prostaglandin analogue in slow release form).

The subsequent menstrual period occurred after a similar interval in each group; the median (range) delay to menstruation was 35 days (24,70), 35 days(26,49) and 32 days (24,48) in the vacuum aspiration, pessary, and device groups respectively. These data are considered in further detail in Chapter 8 in relation to the endocrine profile of the first post-abortion cycle.

Finally, the amount of drug administered to each patient in the controlled-release group was measured by assaying the residual prostaglandin in the device. This was kindly performed by Mr M C Hart, Mr P H Leigh and Dr D J Mills of the Analytical Development

Laboratories, May and Baker Ltd, using high pressure liquid chromatography. The median dose received was 1.65 mg (range 1.16-1.86 mg), representing a percentage release of 55% (range 39% - 62%). There was no relationship between the success rate or the incidence of side effects, and the dose of drug administered.

DISCUSSION

The present work has compared the use of vacuum aspiration with the administration of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester both in pessary form, and incorporated into a controlled-release device in which cross-linking in a strip of polyethylene oxide-based hydrogel is induced by the use of an aliphatic isocyanate (Graham and McNeill, 1984; McNeill and Graham, 1984). On exposure to body fluids, the polymer swells to about three times its dry volume, at a rate which can be predetermined by the composition of the matrix. The drug then diffuses out of the matrix at a zero order rate, over a time period dependent upon the thickness of the device, the degree of crystallinity of the polymer, and the actual prostaglandin used. All of the drug is not released within 24 hours, however measurement of the residual prostaglandin in the devices themselves indicated a reproducible release pattern.

The three treatment groups were comparable in terms of patient characteristics, though only one woman in the smaller device group had carried a previous pregnancy that failed to reach viability.

Both the success rate and the degree of blood loss in the vacuum aspiration group were similar to the findings of previous workers

(Smith and Baird, 1980; Sidhu and Kent, 1984), however the latter study suggested that bleeding may be reduced by pre-operative cervical priming with prostaglandin.

There was no difference between the success rate in the vacuum aspiration group and that of either of the prostaglandin-treated groups.

Furthermore, the onset of bleeding and pain occurred at the same time following both techniques of prostaglandin administration. In addition, although effective abortion was achieved using a reduced dose of drug, there was no significant difference in the observed incidence of gastro-intestinal side effects. This high incidence of prostaglandin-associated gastro-intestinal complications has been noted by others, using a variety of doses and analogues (Bygdeman & Green, 1979) and may reflect the fact that systemic absorption is necessary to achieve the required therapeutic effect. However, although diarrhoea is a relatively specific prostaglandin effect, the occurrence of vomiting may in addition be a consequence of both the use of opiate analgesics, and of the pregnant state itself.

Though there was no difference in analgesic requirements between the two prostaglandin groups, pain relief was required more often in these women than in a previous group treated with the same PG analogue (Smith & Baird, 1980). Direct comparison of such studies should be performed with caution because of differences in the women themselves, and variance in the interpretation of the strength of analgesia needed, both on the part of patients and

nursing staff. Analgesic needs may also be related to the place of treatment, for pain tolerance is likely to be greater in familiar surroundings than in the cold clinical setting of the hospital.

In conclusion, the use of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester in either pessary or slow-release form provided an effective, safe alternative to vacuum aspiration under general anaesthesia, however the reduced dosage administration in the slow release device failed to improve the incidence of gastro-intestinal associated side effects.

It may be that these problems will only be overcome using a different approach to early pregnancy interception. The studies of Csapo illustrating the "prostaglandin impact" and the importance of the removal of progesterone in early abortion have been outlined (see Chapter 5), and the recent development of the antiprogesterone agents (Healy and Fraser, 1985) has presented the opportunity to investigate the role of these compounds as menstrual inducers.

Chapter 7 describes preliminary studies using the progesterone receptor antagonist RU486.

CHAPTER 7

MENSTRUAL INDUCTION USING THE ANTIGESTOGEN RU486

The anti-progesterone agents offer an alternative approach to menstrual induction. Clinical studies have already confirmed the safety of the progesterone-receptor antagonist RU 486, but the low incidence of complete abortion has been disappointing (Herrmann et al, 1982; Kovacs et al, 1984). A combined therapy using both RU 486 and prostaglandin may be more effective, and Swahn et al (1985a) have demonstrated an improved abortion rate using the antigestogen followed by a single intramuscular injection of 16-phenoxy-tetranor-PGE_2 methyl sulfonylamide.

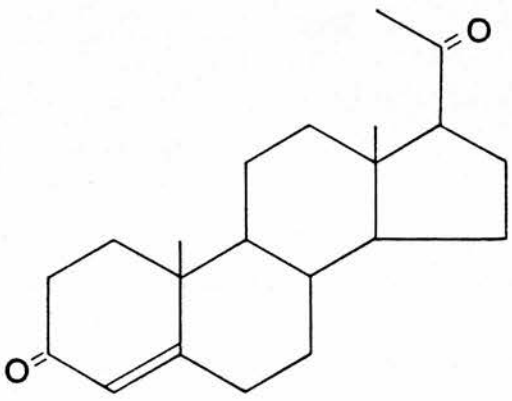
The present study has further investigated the use of RU 486 for the interception of early pregnancy both alone and in combination with gemeprost (16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester) vaginal pessaries.

PATIENTS AND METHODS

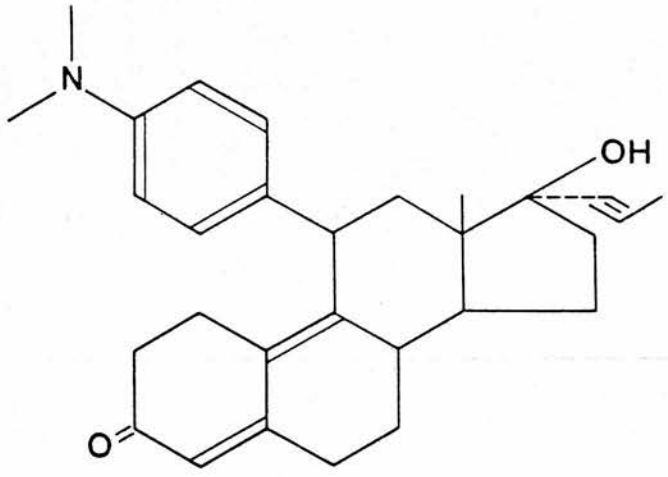
40 women requesting pregnancy termination under the 1967 Abortion Act were recruited for study. All were of a gestational age of 56 days amenorrhoea or less, and all fulfilled the inclusion and exclusion criteria outlined for the comparative study in Chapter 6.

Patients were randomly allocated to the study groups. 20 received RU486 alone (Figure 7), given orally at a dose of 150 mg daily for 4 days. The remainder were also treated with the same dose of RU 486 for 4 days, but in addition they received 1mg of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester in the form of a vaginal pessary given 48 hours after commencing the antigestogen.

The women remained in hospital during drug administration.



PROGESTERONE



RU486

In-patient management and follow up arrangements were as detailed in Chapter 6. Any woman not meeting the clinical and endocrine requirements for a diagnosis of complete abortion at the 2 week follow up visit underwent uterine evacuation under general anaesthesia.

Blood loss was objectively assessed in both groups of women using the alkaline haematin method (Chapter 2).

RESULTS

One woman in the RU486 + PG group had treatment discontinued after 36 hours because of side effects prior to receiving any prostaglandin. She has been excluded from analysis.

There were no differences between the characteristics of the 2 treatment groups (Table 7).

	RU486(20)	RU486+PG(19)
AGE (years)	25(17,41)	23(17,38)
HEIGHT (cm)	168(142,175)	165(157,173)
WEIGHT(kg)	64(47,88)	61(51,68)
PAROUS(%)	5(20%)	2(11%)
GESTATION(days)	47(39,56)	47(35,56)

Table 7 Patient characteristics. Apart from parity, which represents the number of parous women with the percentage in brackets, the data presented show the median and range.

The successful induction of bleeding followed by complete abortion occurred in 12(60%) women in the RU 486 group, but in 18 (95%) of those women treated in combination with PG ($p=0.01$). At uterine evacuation there was evidence of fetal tissue in all 8 treatment

failures in the former group, and in 5 cases the fetal heart was present on ultrasound scanning prior to surgery. The one "failure" in the RU486 + PG group underwent curettage 6 weeks after initial treatment because of persistent mild discharge and an ultrasound appearance consistent with the presence of minimal debris within the uterus. Necrotic trophoblastic tissue was demonstrated histologically at operation.

There were no differences between the two groups in terms of the onset of crampy abdominal pain or vaginal bleeding. The median (range) day of onset was 2(0,4) and 3(0,4) for pain, and 3(1,6) and 3(1,4) for bleeding, in the RU486 alone and RU486 + PG groups respectively. In addition, the use of analgesics during drug administration was similar for the 2 treatment methods, with 15(75%) women in the RU486 group and 10(53%) in the RU486 + PG group requiring no pain relief (N.S. p=0.09). 3(16%) individuals required parenteral analgesia, all of whom were in the PG treated group (N.S. p=0.1).

11 (55%) women in the RU486 group and 7(37%) in the RU486 + PG group complained of nausea during the first 2 days of treatment, whereas in 2(11%) cases in the latter group this was only noticed after PG administration. The incidence of nausea in the 2 groups on the day prior to treatment with RU486 was 58% and 69% respectively in the 13 women whose data were available for analysis.

Vomiting occurred in 1(8%) of 13 women prior to treatment with RU486 alone and in 3(33%) of 9 before they received the combined

therapy. During therapy the number of women with vomiting was 3(15%) and 6(32%) in the RU486 alone and RU486 + PG groups respectively. There was no significant difference between the two groups, nor was there any difference before and after treatment in either group, or in the incidence of vomiting before and after the administration of PG in the combined treatment group.

Diarrhoea was seen on day 2 in one woman in the RU486 alone group, and in one woman on day 3 in the RU486 + PG group.

24 individuals collected their soiled sanitary protection to assess blood loss objectively. The median (range) loss was 53(2,227)mls (n=11) for the RU486 alone group and 81 (32,222) mls (n=13) for the combined RU486 + PG group (N.S. $p>0.05$). Five of the 11 patients in the RU486 alone group had undergone uterine evacuation for failure to induce abortion - the median blood loss of the remaining 6 women was 62(45,227) mls.

One patient in the RU486 alone group had uterine evacuation performed as an emergency because of heavy bleeding 3 days after the cessation of treatment. The degree of blood loss was not measured objectively, however she was not clinically shocked and it was not considered necessary to transfuse her. The patient's haemoglobin concentrations before treatment, and the day after operation, were 12.2 and 11.4 g/dl. Blood transfusion was required though for another woman treated with RU486 alone who bled heavily following uterine evacuation after treatment failure.

Excluding those women who required operative intervention, bleeding persisted for 10(5,29) days in the RU486 alone group and 11(5,34)

days in the PG treated women.

Finally, the interval to the subsequent menstrual period was 39(23,64) days and 35(18,48) days in the RU486 and RU486 +PG groups respectively.

DISCUSSION

The present work has examined the use of RU486 for menstrual induction both alone and in combination with 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester pessaries. Such a combined approach has been advocated on theoretical grounds to improve uterine contractions (Baulieu, 1985a), and in addition the action of the antigestogen to sensitise the uterus to oxytocic agents (Swahn et al, 1985a) should lead to a reduction in the dose of PG required to induce abortion, with a fall in the incidence of unwanted side effects. Using various dose regimes of RU486 in combination with prostaglandin (16-phenoxy-tetranor-PGE_2 methyl sulfonylamide), a complete abortion rate of 100% was seen in 16 women (Swahn et al, 1985a), although a subsequent failure has been reported (Swahn et al, 1985b).

This study has confirmed the success of combined therapy using a fixed dose of RU486, and administering 1mg of synthetic PG in pessary form after 48 hours. A similar success rate for complete abortion would be anticipated with this PG alone (see Chapter 6), but at a dosage of 5 x 1mg pessaries over 12 hours. Although the administration of PG in this form may be more acceptable to the patient herself than intramuscular injection, the latter route may be preferred, as vaginal drug absorption can be altered in the

presence of bleeding. The efficacy of treatment was not impaired in this study though, where 10(53%) women reported the onset of bleeding prior to pessary insertion.

Most patients experienced crampy pain during treatment, and there was no difference between the 2 groups as judged by analgesic requirements. A similar analgesic usage has been reported previously using gemeprost pessaries alone (Smith & Baird, 1980), but more pain relief was required by those 30 women treated with the same PG in Chapter 6 of this thesis; some form of analgesic being requested by 25(86%) women as opposed to 9(47%) for the combined RU486 and PG group in the present work - $p<0.01$. As previously mentioned care must be taken in comparing such results due to the varying subjective awareness of pain in different populations, varying environmental factors, and also to differences in the perception of analgesic requirements on the part of medical and nursing staff.

No serious complications were encountered during therapy, although women in both groups suffered gastro-intestinal associated side effects. Though some patients failed to provide adequate pre-treatment data, it appeared that the incidence of nausea and vomiting did not alter after the commencement of treatment with RU486, nor was it significantly affected by the subsequent administration of PG. As outlined in Chapter 6, nausea and vomiting are commonly seen in early pregnancy, but diarrhoea is a more specific PG-associated problem - this was only seen in one case in each treatment group.

RU486 administration was discontinued in one woman after 36 hours

because of severe nausea and vomiting which pre-dated the start of treatment. She subsequently underwent vacuum aspiration under general anaesthesia without complication, but this emphasises the potential problem of oral drug administration to some women in early pregnancy.

It has been suggested that besides increasing the complete abortion rate, the effect of RU486 plus PG on uterine contractility may also reduce the degree of blood loss (Baulieu, 1985b). This was assessed objectively in the present study; the median values of 52 mls and 81 mls for the 2 groups are comparable with the measured loss following early abortion with either PG alone or vacuum aspiration (Smith & Baird, 1980), and are similar to the expected menstrual loss during a heavy period (Hallberg et al, 1966). 24(61%) individuals actually collected their soiled sanitary protection, but one woman failed to do so because of a complaint of heavy bleeding - the loss was not so heavy as to warrant transfusion or curettage, but her haemoglobin concentration fell from 12.3 g/dl to 11.6 g/dl between the start of treatment and the first follow-up appointment. There was no evidence that the remaining women failed to collect their towels for this reason.

One patient in the RU486 alone group suffered acute bleeding necessitating uterine evacuation 12 hours later. Although the haemorrhage did not require transfusion, and it settled with bed rest, such a conservative approach would not have been clinically acceptable. Previous work has emphasised the potential danger of excessive bleeding after RU486 treatment (Kovacs et al, 1984), however blood loss has not been objectively assessed. Subjective

data on the degree of blood loss between various studies must be interpreted with caution in the light of the finding that up to 50% of women with a subjective complaint of menorrhagia will have a monthly menstrual loss within normal limits (see Chapter 2).

The duration of blood loss was again similar for the 2 treatment groups, and did not differ from that seen previously with either PG or surgically-induced early abortion (Chapter 6).

Thus, the antigestogen RU486 provides a safe method for early abortion, with a low incidence of side effects. When used alone, there is an unacceptably high incomplete or failed abortion rate, however the efficacy of treatment approaches that of standard vacuum aspiration when the drug is administered in conjunction with a low dose of PG. Although further studies are required to assess the most apropriate dosage regimes, such a combined approach to menstrual induction should offer an acceptable alternative to surgery for the interruption of early pregnancy.

CHAPTER 8

THE RETURN TO OVULATION FOLLOWING MENSTRUAL INDUCTION

Although the return of cyclical ovarian function following term delivery has been well studied, the endocrine profile after the interruption of early pregnancy has been less extensively investigated.

It has been known for many years that endometrial regeneration and the return to ovulation is more rapid following abortion than term delivery. Observing the histological changes in the uterine mucosa after birth, Williams (1931) remarked that at least 6 or 7 weeks were required for the disappearance of the placental site in the normal puerperal woman, but Rutherford and Mezer (1942) noted that histological evidence of ovulation occurred within 2 or 3 weeks of early spontaneous abortion.

The return to ovulation after therapeutic termination was also initially studied using endometrial biopsies. Boyd and Holmstrom (1972) examined the endometrium from 72 women on the first or second day of their first menstrual period following induced abortion, and estimated the day of ovulation by subtracting 14 days from the date of the period itself. The mean time to ovulation was 22 days, however there was a wide range (10-72 days). 85% of patients ovulated in the first post-abortion cycle, in contrast to the findings of Reyniak et al (1975) who, also using endometrial biopsies, but at different stages of the menstrual cycle, estimated that only one third of their women ovulated before the first period.

Recently, the hormonal events following pregnancy termination have

been investigated in more detail. Lahteenmaki and Luukkainen (1978) measured plasma estradiol and progesterone concentrations along with the urinary excretion of luteinising hormone (LH) after first trimester termination. They suggested that the refractoriness of ovarian steroidogenesis after abortion was short-lived, with increasing estradiol concentrations from day 8, and 83% of their women ovulated prior to the first menses. Similarly, Blazar et al (1980) assessed the decay of human chorionic gonadotrophin, estradiol and progesterone, along with the changing pattern of pituitary gonadotrophin secretion in two groups of women undergoing vacuum aspiration between 9 and 12 weeks gestation. One group received preoperative cervical ripening with 15 methyl $PGF_{2\alpha}$ methyl ester, and although not using a strict randomisation protocol, this group had a more "normal" endocrine profile, attributed to a possible luteolytic effect of the PG on the corpus luteum of pregnancy.

The present work has investigated the time to ovulation and the hormonal characteristics of the first ovarian cycle following induction of abortion in the first 8 weeks of pregnancy in women allocated to treatment with either vacuum aspiration or 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester pessaries. The return to ovulation has been studied not only as an indicator of the need for early contraception, but also to elucidate the effects of abortion on the synchrony between the ovarian and menstrual cycles.

PATIENTS AND METHODS

32 of the women participating in the comparative study reported in Chapter 6 volunteered to collect urine samples throughout the first

post-abortion cycle. 14 had been treated with vacuum aspiration, whereas the remaining 18 had undergone PG-induced abortion using gemeprost 1mg pessaries. All of the women agreed to use barrier contraception whilst under investigation.

The women were instructed to obtain a sample of first morning urine passed three times weekly, from the time of the induction of abortion until the onset of the subsequent menstrual period. The samples were collected at the routine follow up visits, and were stored frozen until assayed.

Endocrine asays

The measurement of urinary total estrogen, pregnanediol and hCG was carried out by Mr H Boyle and colleagues in the Reproductive Endocrine Laboratories, Centre for Reproductive Biology, University of Edinburgh. The excretion of total estrogen was measured using a fluorometric technique, and pregnanediol was estimated using gas-liquid chromatography, as documented in Chapter 2 (Brown et al, 1968; Chamberlain & Contractor 1968). hCG was assessed using a specific double antibody radioimmunoassay, using reagents kindly supplied by Dr Salvatore Raiti, National Hormone and Pituitary Program, University of Maryland School of Medicine. Details of the assay have been described by Vaitukaitis et al, 1972.
All hormonal parameters were expressed in relation to urinary creatinine (total estrogen-µgm/gm: pregnanediol-mg/gm:hCG-IU/gm).

Ovulation and ovarian function

The day of ovulation was estimated using the change in urinary

pregnanediol excretion in association with changes in total estrogen excretion and a rise in "hCG" (indicating the LH surge) when evident. Ovulation was assumed to have occurred the day prior to that on which the pregnanediol:creatinine ratio first reached 1.0-1.5 mg/gm following the follicular phase. The luteal phase was considered normal if its duration was ⩾11 days (Lenton and Landgren, 1985).

RESULTS

Patient characteristics

The characteristics of the 32 women are shown in Table 8.1. There was no statistical difference between the two groups.

	VACUUM ASPIRATION (14)	PROSTAGLANDIN (18)
AGE (years)	26(19,37)	24(20,37)
HEIGHT(cm)	168(160,175)	170(154,179)
WEIGHT (kg)	59(52,66)	60(50,79)
PARITY >28 weeks	2(14%)	3(17%)
<28 weeks	1(7%)	1(6%)
GESTATION(days)	48(42,55)	49(37,56)

Table 8.1 Patient characteristics. Apart from parity, which is expressed as the number of women, with the percentage in brackets, all data are presented as the median and range.

Abortion was successfully induced in all 32 cases, without the need for subsequent surgical intervention.

Human Chorionic Gonadotrophin

Urinary hCG was measured in 21 women; 9 treated with vacuum

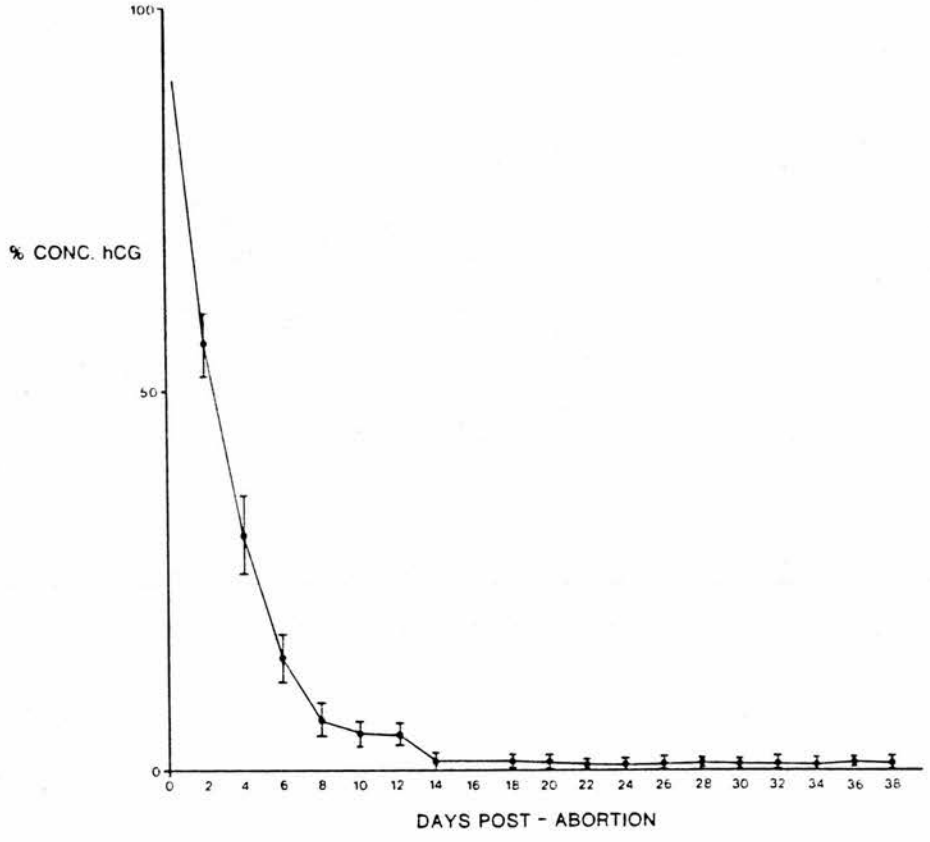
aspiration, and 12 having received PG pessaries. As there was no difference between the two treatment groups in terms of the decay of hCG following abortion, or the return to ovulation (see later), the results have been combined for analysis.

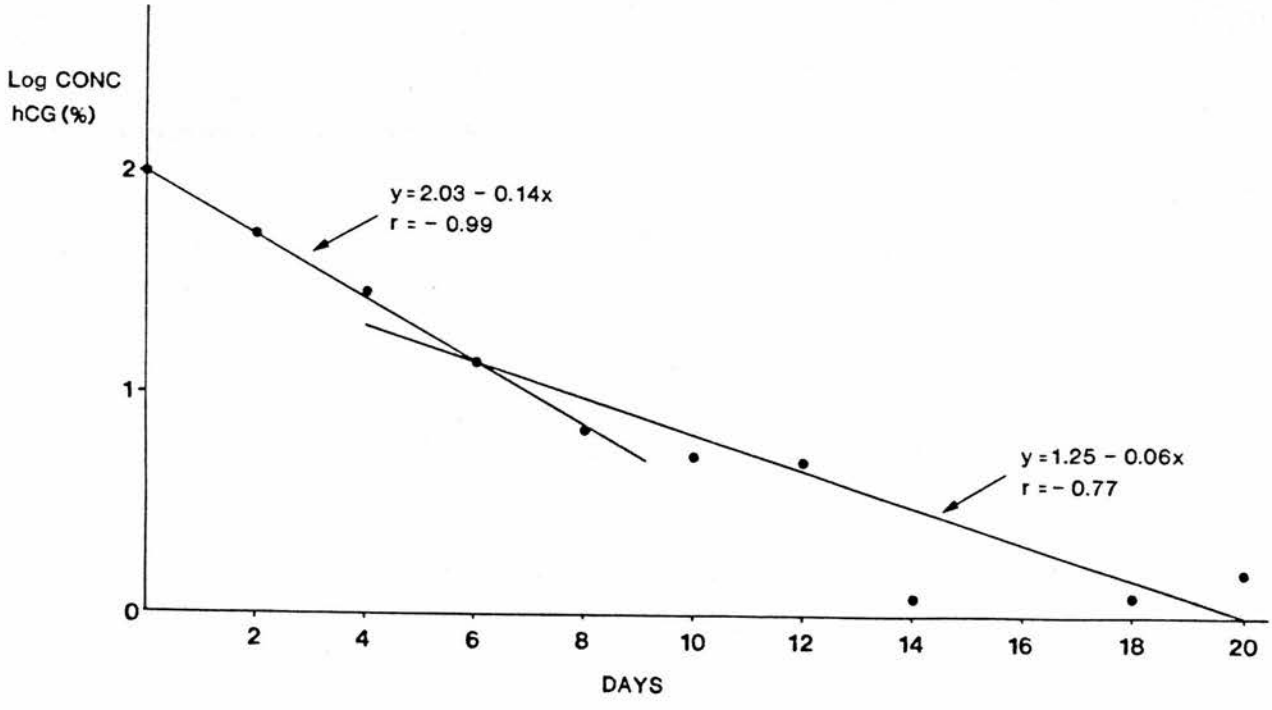
There was a wide range of pre-treatment hCG values (159-200,000 IU/gm) which was not related to gestational age. Figure 8.1a shows the decline of hCG following abortion, expressing the data as percentages of the initial hCG concentration. There was a rapid fall in the excretion of the gonadotrophin, which reached 10% of the pre-treatment value after 6 days in each treatment group (vacuum aspiration-range 2-11 days: PG-range 4-19 days).

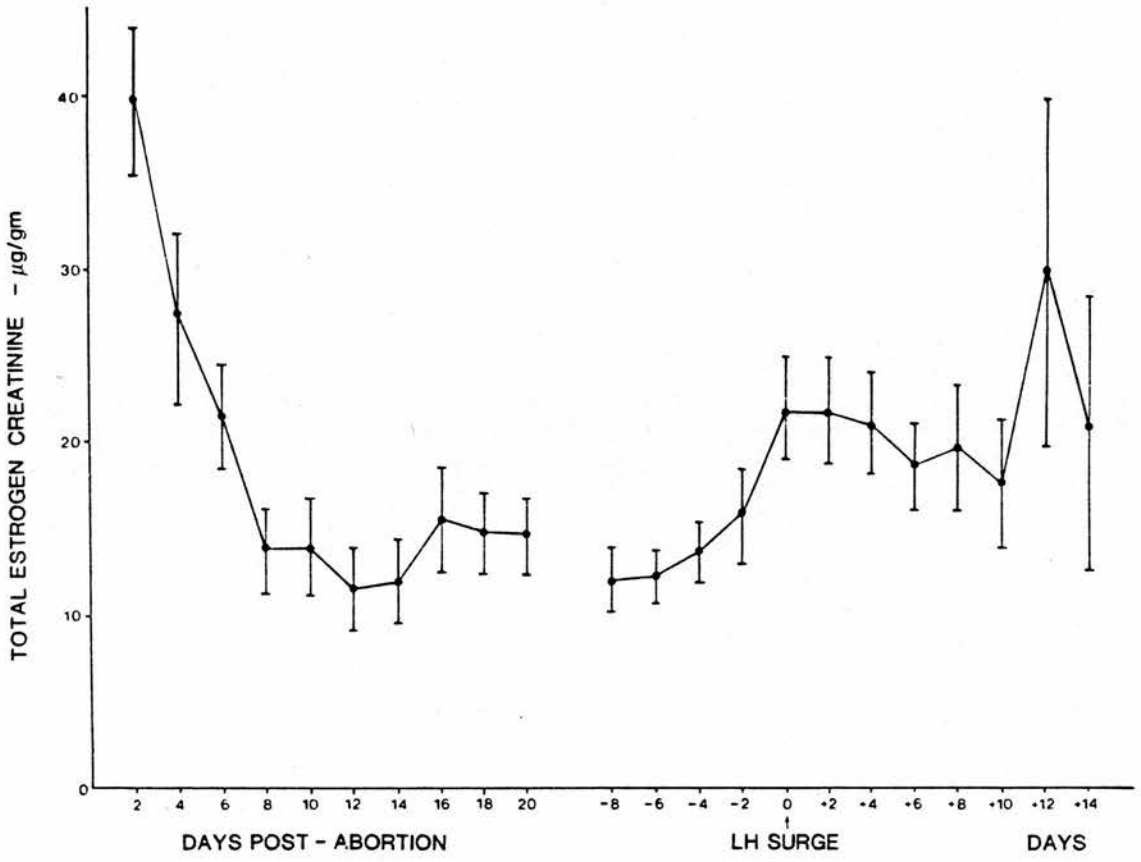
It was not possible to obtain an accurate assessment of the half life($t_{\frac{1}{2}}$) of hCG (the time taken for the concentration of hCG to decline to half of its previous value), due to the infrequency of urine sample collection. However, the hCG decay appeared to consist of an initial rapid component ($t_{\frac{1}{2}}$ ~ 48 hours), followed by a slow component as the excretion of the gonadotrophin fell towards the limit of detection of the assay (figure 8.1b).

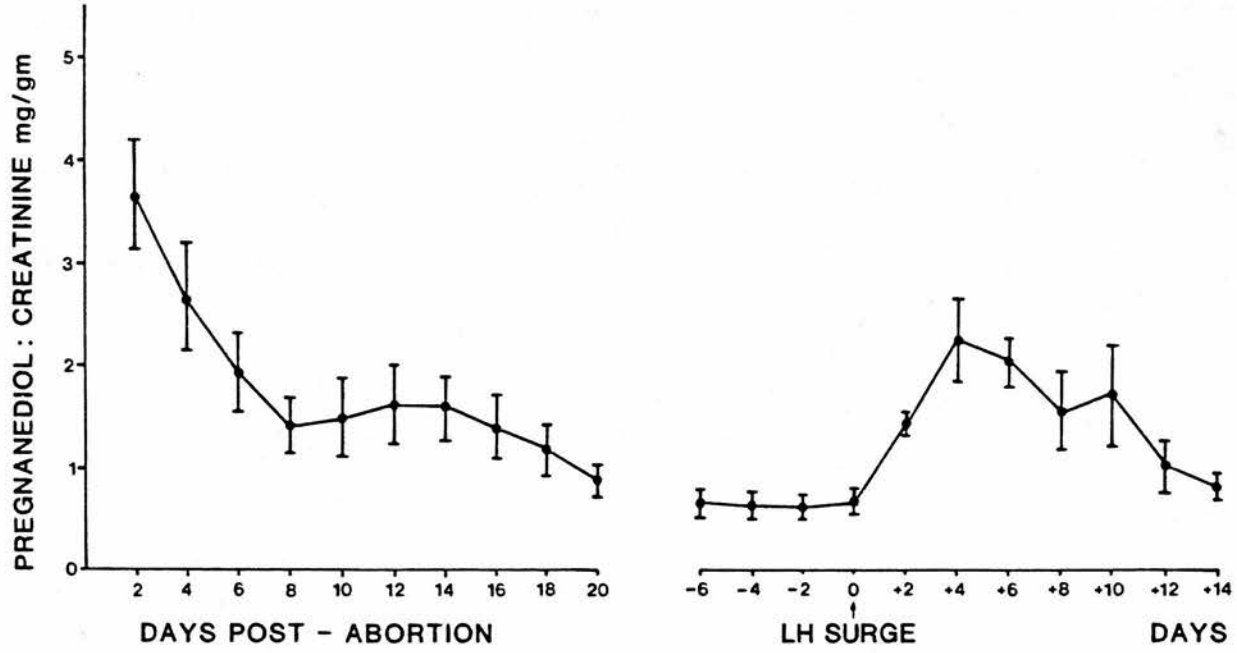
Total estrogen

The changes in urinary total estrogen excretion for the 2 groups combined are presented in figure 8.2a. The initial decline is plotted in relation to the day of abortion, however the data in the latter part of the cycle have been corrected relative to the estimated LH surge (taken as the day prior to the assumed day of ovulation). After a rapid fall in total estrogen excretion, there


% CONC. hCG
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
DAYS POST - ABORTION


Log CONC
hCG (%)
2
1
0
y = 2.03 - 0.14x
r = - 0.99
y = 1.25 - 0.06x
r = - 0.77
2 4 6 8 10 12 14 16 18 20
DAYS


TOTAL ESTROGEN CREATININE - µg/gm
40
30
20
10
0
2 4 6 8 10 12 14 16 18 20
DAYS POST - ABORTION
-8 -6 -4 -2 0 +2 +4 +6 +8 +10 +12 +14
LH SURGE
DAYS


PREGNANEDIOL : CREATININE mg/gm
5
4
3
2
1
0
2 4 6 8 10 12 14 16 18 20
DAYS POST - ABORTION
-6 -4 -2 0 +2 +4 +6 +8 +10 +12 +14
LH SURGE
DAYS

was a gradual increase in the follicular phase, followed by a more rapid rise prior to, and maintained after, ovulation itself. The data from 3 women considered not to have ovulated have been excluded.

Pregnanediol

Figure 8.2b depicts the decline and rise of urinary pregnanediol in both treatment groups similarly plotted relative to abortion itself, and the LH surge.

Return to ovulation

29(91%) women showed a luteal phase rise in pregnanediol excretion, compatible with ovulation. Of the 3 remaining women, 2 had been treated with PG pessaries. All 3 had experienced regular monthly periods prior to abortion.

The time to ovulation is shown in table 8.2. There was no significant difference between the vacuum aspiration and pessary groups in terms of the return to ovulation, but the subsequent period was later in that group receiving operative treatment.

	VACUUM ASPIRATION (13)	PROSTAGLANDIN (16)
DAYS TO OVULATION	29(16,37)	24(16,32)
DAYS TO MENSES	*42(28,49)	*36(26,44)

Table 8.2 The return to ovulation after early abortion. Data are expressed as the median, with the range in parentheses. *$p<0.05$.

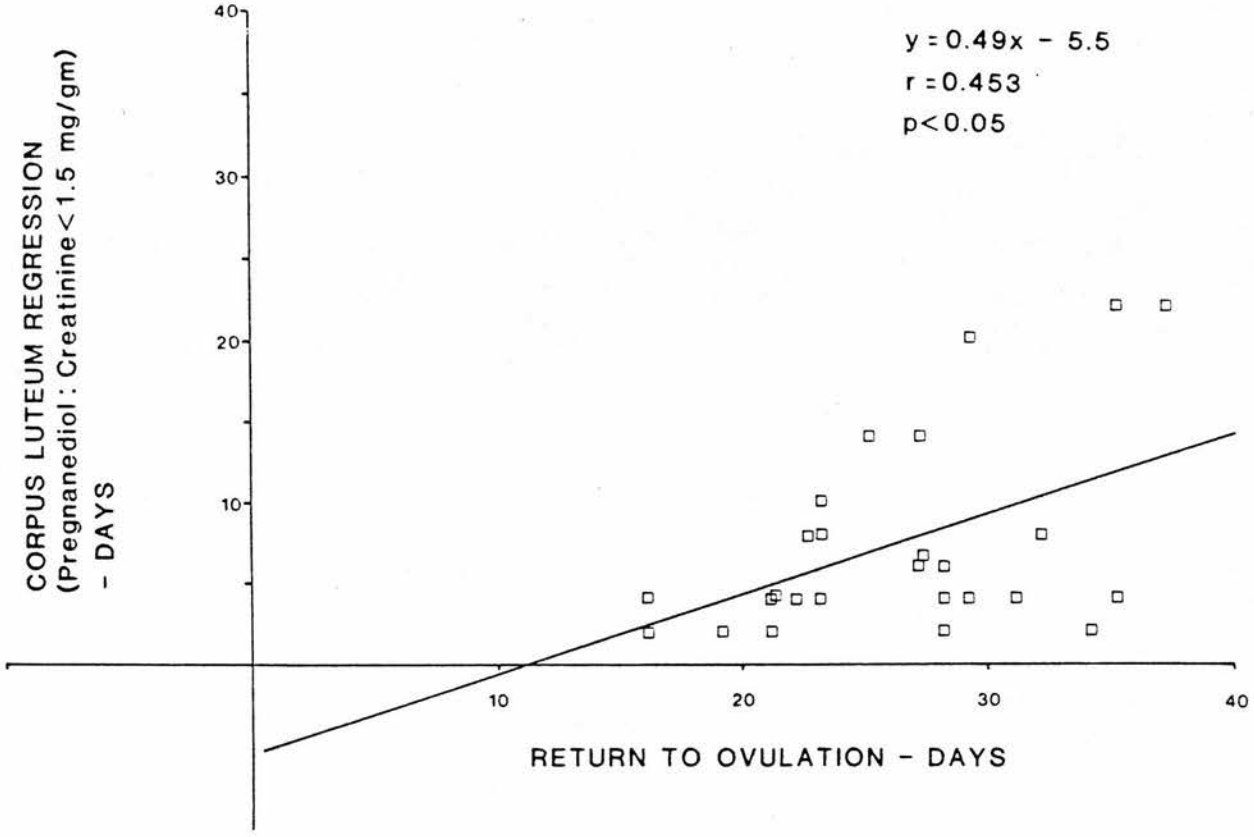
The median length of the luteal phase was similar in both groups (vacuum aspiration - 13 days (range 10-15): PG - 11 days (range 7-14)). However 13(45%) women, 4(30%) in the surgical group and 9(56%) in the PG group, exhibited a luteal phase of <11 days. The difference between the 2 groups was again not statistically significant.

Although the return to ovulation was not directly related to the half life of hCG, there was a correlation between the day on which the urinary pregnanediol: creatinine ratio first fell to less than 1.5 mg/gm, and the day of the return of ovulation (Figure 8.3).

DISCUSSION

The findings of the present study are in accord with previously cited data assessing the endocrine profile of the first post-abortion cycle.

Lahteenmaki and Luukkainen (1978) monitored ovarian function by measuring plasma estradiol and progesterone concentrations in 18 women after first trimester vacuum aspiration. They noted an ovulation rate of 83%, however the luteal progesterone concentration was low compared with previously documented normal cycles. In addition, assessment of the plasma concentrations of hCG/LH and FSH, 3 times weekly, in the same group of subjects (Lahteenmaki, 1978), revealed that although pituitary function recovered within 4-9 days of abortion, the decay of hCG was slow, with complete disappearance occurring after 37.7 days. Blazar et al (1980) similarly investigated the hormonal patterns following early abortion in 15 women undergoing first trimester vacuum aspiration, with 7 of the women receiving pre-operative cervical


y = 0.49x - 5.5
r = 0.453
p < 0.05
CORPUS LUTEUM REGRESSION
(Pregnanediol : Creatinine < 1.5 mg/gm)
- DAYS
40
30
20
10
10
20
30
40
RETURN TO OVULATION - DAYS

ripening using 15 methyl PGE_2 methyl ester pessaries. 13(87%) individuals showed a pre-ovulatory LH peak (7(100%) in the PG group), occurring between day 16 and 35, but as with Lahteenmaki's findings, the subsequent luteal phase progesterone concentrations were low, reaching > 5 ng/ml for 5 days in only 6(40%) individuals, all of whom received pre-operative prostaglandin. The occurrence of more "normal" cycles in the PG-treated group was attributed to either a luteolytic effect, or a specific ovarian action leading to improved follicular development. In the absence of direct evidence for a luteolytic role for the prostaglandins in the human (Baird, 1985), the former hypothesis would seem less likely, and although there was a significant difference between the peak follicular estradiol concentrations in the 2 groups (vacuum aspiration alone - 228 pg/ml: PG-301 pg/ml; $p<0.05$), there was no difference between the groups in relation to the initial decay of estradiol at the time when the PG was administered. However the progesterone concentration declined more rapidly in the PG-treated group (the nadir occurring after 5.6 days as opposed to 7.6 days; $p<0.01$), and it may be that the more "normal" follicular activity is related to the acceleration in the rate of decay of progesterone concentrations.

Although the changes in urinary steroids and hCG in the present work are similar to these previous studies, the use of urine rather than plasma results in differences in the interpretation of multi-component hormonal decay curves. For example, the initial rapid component decay of hCG in Lahteenmaki's work ($t_{\frac{1}{2}}$ ~ 15hrs) represents disappearance of the gonadotrophin from plasma. Though an accurate estimate of hCG $t_{\frac{1}{2}}$ was not obtained here, the development of subsequent ovarian function and ovulation occurred

in the presence of detectable hCG, as previously reported (Lahteenmaki, 1978; Blazar et al, 1980).

An ovulation rate of 91% compares well with previous studies using both histological and endocrine methods, but although the luteal phase was short in 13(45%) women, the relatively infrequent urine sampling has prevented more specific comment regarding luteal function. Regardless of this, the high ovulation rate in the first cycle post-abortion demands the use of reliable contraception from an early stage.

The return to ovulation on day 24 and day 29 in the PG and vacuum aspiration groups respectively, also agrees with previous findings, however, there was a wide range (16-37 days). This delay to ovulation was not related to the decline ($t_{\frac{1}{2}}$) of hCG, but correlated with the day on which the urinary pregnanediol:creatinine ratio first fell below 1.5 mg/gm. As this would represent progesterone from both the corpus luteum and the products of conception, and the hCG would mirror remaining trophoblastic activity, it is likely that delay in the decline of pregnanediol:creatinine is related to a delay in luteolysis. Once the corpus luteum's progesterone-secreting activity has been overcome, the return of pituitary gonadotrophin release, even in the presence of some hCG, would result in the resumption of cyclical ovarian activity. Assuming a normal follicular phase, the delay in the occurrence of ovulation, as the data suggest, would be directly related to delay in the initiation of follicular activity.

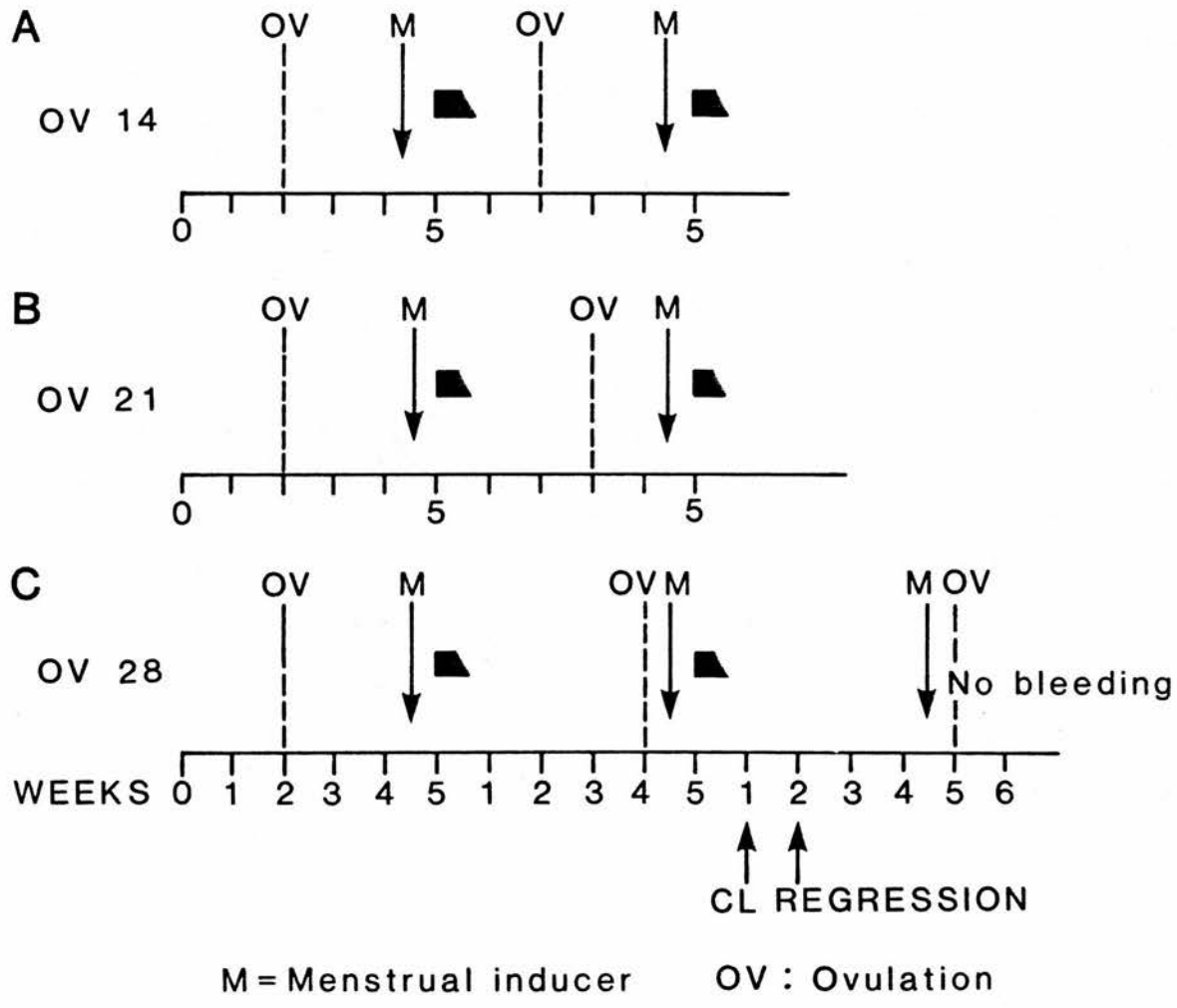
Delay in luteolysis may result from continued support by hCG. This

residual hCG activity will derive from both its long half-life, and any remaining trophoblast. The present data do not allow the relative contributions of these two factors to be separated, however there was no difference between the two treatment groups. In addition, the part played by the hCG half-life will depend on the initial concentration of hCG - which will result in varying absolute concentrations of hCG after any unit of time - and the sensitivity of the individual corpus luteum to the gonadotrophin itself.

In conclusion, most women ovulated in the first post-abortion cycle and there was no difference between the 2 treatment groups. However, the return to ovulation occurred over a wide range, and this was related to the decline of pregnancy corpus luteum function. What then is the clinical significance of this delay to ovulation?

The need for early reliable contraception has already been mentioned, but if menstrual induction is to be considered as a routine method of fertility control, it is necessary to discuss the effect of the delay to ovulation on the synchrony between the ovarian and menstrual cycles.

The problem of repeated menstrual induction is outlined in figure 8.4. In A, ovulation (OV) occurs 14 days after induction of menses with a "menstrual inducer" (M) in month 2, and M is taken for the second time at the appropriate stage. In B, the menstrual inducer is merely taken earlier in the secretory phase. If, however, ovulation is delayed by 2 weeks after induction of abortion in


A
OV
M
OV
M
OV 14
0
5
5
B
OV
M
OV
M
OV 21
0
5
5
C
OV
M
OV
M
M
OV
OV 28
No bleeding
WEEKS 0 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 6
CL REGRESSION
M = Menstrual inducer
OV : Ovulation

cycle 1, and this occurred in 11(38%) women in the present study (see C), the menstrual inducer is taken progressively earlier in the cycle, and by cycle 3 it is taken in the follicular phase. No bleeding will ensue and the subject will assume that she is still pregnant. This disruption of the synchrony between the ovarian and menstrual cycles represents a major constraint to the use of early pregnancy interruption as a long term method of fertility control, however, further studies are required to assess whether these problems could be overcome by administering therapy in very early pregnancy.

PROSTAGLANDINS AND MENSTRUAL INDUCTION: CONCLUSION

The clinical potential of the prostaglandins as agents for menstrual induction offers a practical alternative to vacuum aspiration. The effectiveness of one such agent has been described both alone (Chapter 6) and in combination with RU486 (Chapter 7), however, the widespread application of medical abortion remains limited.

Firstly, the abortion process is not immediate, as opposed to complete vacuum aspiration, and takes place over some days. Many women prefer the more expeditious surgical option, usually performed on an out-patient basis. Secondly, in the absence of a treatment method with a reliability approaching 100%, a high degree of patient compliance is required, particularly in relation to follow up. At operation the uterine contents can be examined to confirm expulsion of the fetus. This is not practically possible using medical agents for early termination, and makes follow up mandatory to audit treatment success. In addition, the inability to confirm the removal of fetal and trophoblastic tissue from the uterine cavity prevents the exclusion of ectopic gestation. The delayed diagnosis of ectopic pregnancy in a woman undergoing menstrual induction using RU486 has been reported (Kovacs et al, 1984), and in the present work one patient referred for the comparative study in Chapter 6 had an ovarian ectopic gestation. She had conceived with an intra-uterine contraceptive device in situ, and her only presenting complaint had been menstrual spotting for a week, which had excluded her from the study itself. Although the diagnosis of intra-uterine pregnancy can be made by ultrasound, this is less reliable in very early pregnancy, in the absence of a definite fetal pole or fetal heart. The possibility of ectopic pregnancy should always be considered, especially in those

situations where associated predisposing factors for ectopic implantation exist.

The other major drawbacks to the acceptability of medical agents for menstrual induction are the occurrence of side effects, and the disturbed synchrony between the ovarian and menstrual cycles (Chapter 8). The attempted improvement in gastro-intestinal side effects by the administration of a low dose of PG in a controlled release fashion was unsuccessful (Chapter 6), however this approach should be further investigated using different PG analogues. Slow-release drug administration could also be applied in other situations, such as the induction of second trimester abortion (Bygdeman et al, 1984), the management of intrauterine death, and the induction of labour (Greer et al, 1986).

A combination of a low dose of PG with RU486 achieved an acceptable abortion rate with a partial improvement in gastro-intestinal effects (Chapter 7), though nausea and vomiting continued to occur. It may be that the incidence of these side effects will not be improved in early pregnancy without resorting to specific antiemetic premedication.

The delay to ovulation in some women following menstrual induction with PG or vacuum aspiration could result in asynchrony between the ovarian and menstrual cycles if treatment were prescribed on a regular basis (Chapter 8). This problem might be overcome by administering the menstrual inducer in very early pregnancy, or by using combination drug therapy, however, a delay to ovulation of 32 days has been noted using luteal phase menstrual induction with RU486 in rhesus monkeys (Hodgen, 1985).

In view of the increasing interest in medical agents for menstrual induction (Rosen et al, 1979), much further research is required in this field of fertility control.

REFERENCES

Abel MH, Baird, DT (1980). The effect of 17ß estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinology 106, 1599-1606.

Abel MH, Kelly RW (1979). Differential production of prostaglandins within the human uterus. Prostaglandins 18, 821-828.

Abel MH, Kelly RW (1983). Metabolism of prostaglandins by the non pregnant human uterus. Journal of Clinical Endocrinology & Metabolism 56, 678-685.

Aegostina T, Sastrawinata S, Prayitno W, Gonta P (1984). Menstrual regulation with prostaglandin ONO-802 in Indonesia. International Journal of Gynaecology & Obstetrics 22, 403-409.

Allen WM, Corner GW (1930). Physiology of corpus luteum. VII. Maintenance of pregnancy in rabbit after very early castration, by corpus luteum extracts. Proceedings of the Society for Experimental Biology & Medicine 27, 403-405.

Anderson ABM, Haynes PJ, Guillebaud J, Turnbull AC (1976). Reduction of menstrual blood loss by prostaglandin synthetase inhibitors. Lancet i., 774-776.

Baird DT (1985). Control of luteolysis. In: The luteal phase. Editor: Jeffcoate SL, John Wiley & Sons, Ltd. U.K. pp 25-42.

Baird DT, Abel MH, Kelly RW, Smith SK (1981). Endocrinology of dysfunctional uterine bleeding: the role of endometrial prostaglandins. In: Endocrinology of Human Infertility: New Aspects. Serono clinical colloquia on Reproduction. 2. Editors: Crosignani PG, Rubin BL Academic Press. London. pp 399-417.

Baird DT, Cameron IT (1985). Menstrual induction: surgery versus prostaglandins. In: Abortion: medical progress and social implications. (CIBA Foundation Symposium 115) John Wiley & Sons Ltd, U.K., pp 178-191.

Barer AP, Fowler WM (1936). The blood loss during normal menstruation. American Journal of Obstetrics & Gynecology 31, 979-986.

Baulieu E-E (1985a). RU486: an antiprogestin steroid with contragestive activity in women. In: The antiprogestin steroid RU486 and human fertility control. Editors: Baulieu E-E, Segal SJ Plenum Press, New York. pp 1-25.

Baulieu E-E (1985b) Contragestion by antiprogestin: a new approach to human fertility control. In: Abortion: medical progress and social implications. (CIBA Foundation Symposium 115) John Wiley & Sons Ltd, U.K. pp 192-210.

Beckwith Whitehouse H (1914). The physiology and pathology of uterine haemorrhage. Lancet i, 877-885 and 951-957.

Berqvist A, Rybo G (1983). Treatment of menorrhagia with

intrauterine release of progesterone. British Journal of Obstetrics & Gynaecology 90, 255-258.

Bevan JR, Dowsett M, Jeffcoate SL (1984). Endocrine effects of Danazol in the treatment of endometriosis. British Journal of Obstetrics & Gynaecology 91, 160-166.

Bishop PMF, de Almeida JCC (1960). Treatment of functional menstrual disorders with norethisterone. British Medical Journal 1, 1103-1105.

Bito LZ (1975). Are prostaglandins intracellular, transcellular or extracellular autocoids? Prostaglandins 9,851-855.

Blatchley FR, Poyser NL (1974). The effect of estrogen and progesterone on the release of prostaglandins from the uterus of the ovariectomised guinea-pig. Journal of Reproduction & Fertility 40, 205-209.

Blazar AS, Harlin J, Zaidi A, Diczfalusy E. (1980). Differences in hormonal patterns during the first post-abortion menstrual cycle after two techniques of termination of pregnancy. Fertility & Sterility 33, 493-500.

Bonnar J, Sheppard BL (1979). Endometrial changes in women using hormone-releasing intra-uterine devices. In: WHO Symposium on steroid contraception and mechanisms of endometrial bleeding. Geneva, September 1979. pp. 347-365.

Bonnar J, Sheppard BL, Dockeray CJ (1983). The haemostatic system and dysfunctional uterine bleeding. Research and Clinical Forums 5, 27-38.

Boyd EF, Holstrom E. (1972). Ovulation following therapeutic abortion. American Journal of Obstetrics and Gynecology 113, 469-473.

Brennecke SP, Castle BM, Demers LM, Turnbull AC. (1985). Maternal plasma prostaglandin E_2 metabolite levels during human pregnancy and parturition. British Journal of Obstetrics & Gynaecology 92, 345-349.

Brown JB, Macleod SL, MacNaughton C, Smith MA, Smith B. (1968). A rapid method for estimating oestrogens in urine using a semi-automated extractor. Journal of Endocrinology 42, 5-15.

Bryant-Greenwood GD, Greenwood FC, Hale RW, Morishige WK (1977). Hormonal evaluation of the intrauterine progesterone contraceptive system (IPCS). Journal of Clinical Endocrinology & Metabolism. 44, 721-727.

Bygdeman M, Bremme K, Christensen N, Lundstrom V, Green K (1980). A comparison of two stable prostaglandin E analogues for termination of early pregnancy and for cervical dilatation. Contraception 22, 471-483.

Bygdeman M, Christensen N, Dimov V, Green K. (1984). Termination of pregnancy by a slow release device containing 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester. Asia-Oceana Journal of Obstetrics & Gynaecology 10, 359-365.

Bygdeman M, Green K. (1979). Menstrual regulation: medical techniques. In: Pregnancy Termination - Procedures, Safety and New Developments. Editors: Zatuchni GI, Sciarra JJ, Speidal JJ Harper & Rowe. pp 69-77.

Cameron IT, Baird DT. (1984). The use of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester (gemeprost) vaginal pessaries for the termination of pregnancy in the early second trimester. A comparison with extra-amniotic prostaglandin E_2. British Journal of Obstetrics & Gynaecology 91, 1136-1140.

Casey ML, Hensell DL, MacDonald PC, Johnson JM. (1980) NAD^+ dependent 15 hydroxyprostaglandin dehydrogenase activity in human endometrium. Prostaglandins 19, 115-122.

Chamberlain J, Contractor SF (1968). A gas liquid chromatographic method for the rapid estimation of pregnanediol and allopregnanediol in non-pregnancy urine. American Journal of Obstetrics & Gynecology 101, 649-657.

Chan WY, Hill JC (1978). Determination of menstrual prostaglandin levels in non-dysmenorrhoeic and dysmenorrhoeic subjects. Prostaglandins 15, 365-375.

Chimbira TG, Anderson ABM, Naish C, Cope E, Turnbull AC (1980). Reduction of menstrual blood loss by danazol in unexplained menorrhagia: lack of effect of placebo. British Journal of Obstetrics & Gynaecology 87, 1152-1158.

Chimbira TG, Cope E, Anderson ABM, Bolton FG (1979). The effect of danazol on menorrhagia, coagulation mechanisms, haematological indices and body weight. British Journal of Obstetrics & Gynaecology 86, 46-50.

Cohen BJB, Gibor Y (1980). Anaemia and menstrual blood loss. Obstetrical & Gynecological Survey 35, 597-618.

Cohen BJB, Gibor Y (1983). Metabolic and endocrine effects of medicated intra-uterine devices. In: Endocrine mechanisms in Fertility Regulation. Editors: Benagiano G, Diczfalusy E, Raven Press, New York pp 71-124.

Cole SK, Billewicz WZ, Thomson AM (1971). Sources of variation in menstrual blood loss. Journal of Obstetrics & Gynaecology of the British Commonwealth 78, 933-939.

Colle ML, Greenblatt RB (1976). Contraceptive properties of Danazol. Journal of Reproductive Medicine 17, 98-102.

Csapo AI, Herczeg J, Pulkkinen MO, Kaihola HL, Zoltan I, Csillag M, Mocsary P (1976). Termination of pregnancy with double prostaglandin impact. American Journal of Obstetrics & Gynecology 124, 1-13.

Csapo AI, Mocsary P, Nagy T, Kaihola HL (1973a). The efficacy and acceptability of the "prostaglandin impact" in inducing complete abortion during the second week after the missed menstrual period. Prostaglandins 3, 125-139.

Csapo AI, Peskin EG, Pulkkinen MO, Laajoki V, Kivikoski A, Compe L, Godeny S, Szeverenyi M, Herczeg J, Resch B, Bacos L (1982). Menstrual induction with sulproston. Prostaglandins 24, 657-665.

Csapo AI, Pulkkinen MO (1979a). The mechanism of prostaglandin action on the pregnant human uterus. Prostaglandins 17, 283-299.

Csapo AI, Pulkkinen MO (1979b). The mechanism of prostaglandin action on the early pregnant human uterus. Prostaglandins 18, 479-490.

Csapo AI, Pulkkinen MO, Wiest NG (1973b). Effects of luteectomy and progesterone replacement therapy in early pregnant patients. American Journal of Obstetrics & Gynecology 115, 759-765.

Demers LM, Brennecke SP, Mountford LA, Brunt JD, Turnbull AC (1983). Development and validation of a radioimmunoassay for prostaglandin E_2 metabolite levels in plasma. Journal of Clinical Endocrinology & Metabolism 57, 101-106.

Demers LM, Rees MCP, Turnbull AC (1984). Arachidonic acid metabolism by the non-pregnant human uterus. Prostaglandins, leukotrienes & medicine 14, 175-180.

Dimov V, Christensen NJ, Green K (1983). Analysis of prostaglandins formed from endogenous and exogenous arachidonic acid in homogenates of human reproductive tissues. Biochemica et Biophysica Acta 754, 38-43.

Dmowski WC, (1979). Endocrine properties and clinical application of danazol. Fertility & Sterility 31, 237-248.

Dockeray CJ, Sheppard BL, Bonnar J (1986). The efficacy of mefenamic acid and danazol in the treatment of established menorrhagia. 24th. British Congress of Obstetrics & Gynaecology, Cardiff, April 1986. Abst. 150.

Downie J, Poyser NL, Wunderlich M (1974). Levels of prostaglandins in human endometrium during the normal menstrual cycle. Journal of Physiology 236, 465-472.

Edelman DA, Berger GS (1981). Menstrual Regulation. In: Abortion and sterilisation: medical and social aspects. Editor: Hodgson JE, Academic Press. London pp. 209-224.

Embrey MP, Graham NB, McNeill ME (1980). Induction of labour with a sustained-release prostaglandin E_2 vaginal pessary. British Medical Journal 281, 901-902.

Fenwick L, Jones RK, Naylor N, Poyser NL, Wilson NH (1977). Production of prostaglandins by pseudopregnant rat uterus, in vitro, and effect of tamoxifen, with the identification of 6ketoPGF$_{1\alpha}$ as a major product. British Journal of Pharmacology 59, 191-199.

Ferreira SH, Moncada S, Vane JR (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature. New Biology 231, 237-239.

Flower RJ (1980). Phospholipases and their relevance to prostaglandin biosynthesis. In: The prostaglandin system. Editors: Benti F & Velo GP, Plenum Press. New York. pp. 27-37.

Foster HW, Smith M, McGruder CE, Richard F, McIntyre J (1985). Postconception menses induction using prostaglandin vaginal suppositories. Obstetrics & Gynecology,65, 682-685.

Fraser HM (1981). Luteinising hormone-releasing hormone and fertility control. In: Oxford Reviews of Reproductive Biology, Editor: Finn CA, Oxford University Press. 3, 1-48.

Fraser IS (1983). The treatment of menorrhagia with mefenamic acid. Research & Clinical Forums 5, 93-102.

Fraser IS, McCarron G, Markham R (1984). A preliminary study of factors influencing perception of menstrual blood loss volume. American Journal of Obstetrics & Gynecology 149, 788-793.

Fraser IS, McCarron G, Markham R, Resta T (1985). Blood and total fluid content of menstrual discharge. Obstetrics & Gynecology 65, 194-198.

Fraser IS, Michie EA, Wide L, Baird DT (1973). Pituitary gonadotrophins and ovarian function in adolescent dysfunctional uterine bleeding. Journal of Clinical Endocrinology & Metabolism 37, 407-414.

Fraser IS, Pearse C, Shearman RP, Elliott PM, McIlveen J, Markham R (1981). Efficacy of mefenamic acid in patients with a complaint of menorrhagia. Obstetrics & Gynecology 58, 543-551.

Graham NB, McNeill ME (1984). Hydrogels for controlled drug delivery. Biomaterials 5, 27-36.

Granstrom E, Kindahal H (1982). Radioimmunoassay of 5 ,7 -dihydroxy-11-ketoefranorprostane-1,16-dioic acid, a major prostaglandin F metabolite in blood and urine. Methods in Enzymology 86, 339-353.

Green K (1979). Determination of prostaglandins in body fluids and tissues. Acta Obstetrica et Gynaecologica Scandinavica Suppl. 87, 15-20.

Green K, Hagenfeldt K (1975). Prostaglandins in the human endometrium. GC-MS quantitation before and after IUD insertion. American Journal of Obstetrics & Gynecology 122, 611-614.

Greenblatt RB, Oettinger M, Borenstein R, Bohler CS-S (1974). Influence of danazol (100mg) on conception and contraception. Journal of Reproductive Medicine 13, 201-203.

Greer IA, Smith JR, Godfree V, McLaren M, Graham N, Calder AA (1986). PGE_2 absorption after slow release PGE_2 pessaries for the induction of labour. 24th British Congress of Obstetrics & Gynaecology, Cardiff. April 1986. Abst. 237.

Gryglewski RJ, Bunting S, Moncada S, Flower RJ, Vane JR (1976).

Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin x) which they make from prostaglandin endoperoxides. Prostaglandins 12, 685-713.

Guillebaud J, Anderson ABM, Turnbull AC (1978). Reduction by mefenamic acid of increased menstrual blood loss associated with intrauterine contraception. British Journal of Obstetrics & Gynaecology 85, 53-62.

Gurpide E, Markiewicz L, Schatz F, Hirata F (1986). Lipocortin output by human endometrium in vitro. Journal of Clinical Endocrinology & Metabolism 63, 162-166.

Hagenfeldt K, Landgren B-M, Edstrom K, Johannisson E (1977). Biochemical and morphological changes in the human endometrium induced by the progestasert device. Contraception 16, 183-197.

Hahn DW, McGuire JL, Carraher RP, Demers LM (1985). Influence of ovarian steroids on prostaglandin - and leukotriene - induced uterine contractions. American Journal of Obstetrics & Gynecology 153, 87-91.

Hallberg L, Hogdahl AM, Nilsson L, Rybo G (1966). Menstrual blood loss - a population study. Acta Obstetrica et Gynaecologica Scandinavica 45, 320-351.

Hallberg L, Nilsson L, (1964). Determination of menstrual blood loss. Scandinavian Journal of Clinical and Laboratory Investigation 16, 244-248.

Ham EA, Cirillo VJ, Zanetti ME, Kuehl FA (1975). Estrogen-directed synthesis of specific prostaglandins in uterus. Proceedings of the National Academy of Sciences, USA 72, 1420-1424.

Harrison RF, Campbell S (1976). A double blind trial of ethamsylate in the treatment of primary and intrauterine-device menorrhagia. Lancet ii, 283-285.

Haynes PJ, Anderson ABM, Turnbull AC (1979). Patterns of menstrual blood loss in menorrhagia. Research and Clinical Forums. 1, 73-78.

Haynes PJ, Hodgson H, Anderson ABM, Turnbull AC (1977). Measurement of menstrual blood loss in patients complaining of menorrhagia. British Journal of Obstetrics & Gynaecology 84, 763-768.

Healy DL, Cameron IT (1987). Clinical Physiology of the endometrium. In: Reproductive Physiology in clinical practice. Editor: Riddick D, Thieme Stratton. New York. pp 51-70.

Healy DL, Fraser HM (1985) The antiprogesterones are coming: menses induction, abortion, and labour? British Medical Journal 290, 580-581.

Hern WM (1984). Operative procedures and techniques. In: Abortion practice. Lippincott. Philadelphia. pp. 101-160.

Herrmann W, Wyss R, Riondel A, Philibert D, Teutsch G, Sakiz E, Baulieu E-E (1982). The effects of an antiprogesterone steroid in women: Interruption of the menstrual cycle and of early pregnancy. Comptes Rendus 294, 933-940.

Hillier K, Kasonde JM (1976). PGE and F concentrations in human endometrium after insertion of IUCD. Lancet i, 15-16.

Hobson BM (1968). Pregnancy diagnosis using the pregnosticon haemagglutination inhibition test. British Journal of Obstetrics & Gynaecology. 75, 718-723.

Hodgen GD (1985). Pregnancy prevention by intravaginal delivery of a progesterone antagonist: RU486 tampon for menstrual induction and absorption. Fertility & Sterility 44, 263-267.

Hoppe-Seyler G, Brodersen, Rudolph (1904). Uber den blutverlust bei der menstruation. Zeitschrift fur physiologische chemie 42, 545-553.

Horton EW, Poyser NL (1976). Uterine luteolytic hormone: a physiological role for prostaglandin $F_{2\alpha}$. Physiological Reviews 56, 913-919.

Jacobs A, Butler EB (1965). Menstrual blood loss in iron deficiency anaemia. Lancet ii, 407-409.

Janne O, Kontula K, Luukkainen T, Vihko R (1975). Oestrogen induced progesterone receptor in human uterus. Journal of steroid biochemistry 6, 501-509.

Johnstone FD, Beard RJ, Boyd IE, McCarthy TG (1976). Cervical diameter after suction termination of pregnancy. British Medical Journal 1, 68-69.

Jones RK, Poyser NL, Wilson NH (1977). Production of 6oxoPGF$_{1\alpha}$ by rat, guinea-pig and sheep uteri in vitro. British Journal of Pharmacology. 59, 436-437.

Karim SMM (1971). Once-a-month vaginal administration of prostaglandins E_2 and $F_{2\alpha}$for fertility control. Contraception 3, 173-183.

Karim SMM, Filshie GM (1970). Therapeutic abortion using prostaglandin $F_{2\alpha}$. Lancet i, 157-159.

Karman H, Potts M (1972). Very early abortion using syringe as vacuum source. Lancet i, 1051-1052.

Kelly RW, Abel MH (1983) The measurement of 13,14-dihydro-15-keto prostaglandin E_2 by combined gas chromatography mass spectrometry. Biomedical mass spectrometry 10, 276-279.

Kelly RW, Deam S, Cameron MJ, Seamark RF (1986a). Measurement by radioimmunoassay of prostaglandins as their methyl oximes. Prostaglandins, leukotrienes and medicine (in press).

Kelly RW, Healy DL, Cameron MJ, Cameron IT, Baird DT (1986b). The stimulation of prostaglandin production by two antiprogesterone steroids in human endometrial cells. Journal of Clinical Endocrinology & Metabolism 62, 1116-1123.

Kelly RW, Lumsden MA, Abel MH, Baird DT (1984). The relationship

between menstrual blood loss and prostaglandin production in the human: evidence for increased availability of arachidonic acid in women suffering from menorrhagia. Prostaglandins, leukotrienes and medicine 16, 69-78.

Kovacs L, Sas M, Resch BA, Ugocsai G, Swahn ML, Bygdeman M, Rowe PJ (1984). Termination of very early pregnancy by RU486 - an antiprogestational compound. Contraception 29, 399-410.

Lahteenmaki P (1978). The disappearance of hCG and return of pituitary function after abortion. Clinical Endocrinology 9, 101-112.

Lahteenmaki P, Luukkainen T (1978). Return of ovarian function after abortion. Clinical Endocrinology 8, 123-132.

Larsson-Cohn J, Johansson EDB, Gemzell C (1971). Effects of continuous daily administration of 0.03 mg of d-norgestrel on the plasma levels of progesterone and the urinary excretion of androgens. Acta Endocrinologica 66, 702-710.

Lenton EA, Landgren BM (1985). The normal menstrual cycle. In: Clinical Reproductive Endocrinology. Editor: Shearman RP, Churchill Livingstone. pp. 81-108.

Liggins GC, Campos GA, Roberts CM, Skinner SJ (1980). Production rates of prostaglandin F, 6keto-$PGF_{1\alpha}$ and thromboxane B_2 by perifused human endometrium. Prostaglandins 19, 461-477.

Lumsden MA, Kelly RW, Baird DT (1983). Is prostaglandin $F_{2\alpha}$ involved in the increased myometrial contractility of primary dysmenorrhoea? Prostaglandins 25, 683-692.

Maathius H, Kelly RW (1978). Concentrations of prostaglandins $F_{2\alpha}$ and E_2 in the endometrium throughout the human menstrual cycle, after the administration of clomiphene or an oestrogen-progesterone pill, and in early pregnancy. Journal of Endocrinology 77, 361-371.

MacKenzie IZ, Embrey MP, Davies AJ, Guillebaud J (1978). Very early abortion by prostaglandins. Lancet i., 1223-1226.

Malik KV, McGiff JC (1976). Cardiovascular action of prostaglandins. In: Prostaglandins: physiological, pharmacological and pathological aspects. Editor: Karim, SMM, MTP Press Ltd. Lancaster pp. 103-200.

Markee JE (1940). Menstruation in intraocular endometrial transplants in the rhesus monkey. Contributions to Embryology 28, 219-308.

McNeill ME, Graham NB (1984). Vaginal pessaries for crystalline/rubbery hydrogels for the delivery of prostaglandin E_2. Journal of Controlled Release 1, 99-117.

Meyer JH (1983). Early office termination of pregnancy by soft cannula vacuum aspiration. American Journal of Obstetrics & Gynecology 147, 202-207.

Mitchell S, Poyser NL, Wilson NH (1977). Effect of p-bromophenacyl bromide, an inhibitor of phospholipase A_2, on arachidonic acid release and prostaglandin synthesis by guinea-pig uterus in vitro. British Journal of Pharmacology 59, 107-113.

Moncada S, Bunting S, Mullane K, Thorogood P, Vane JR, Raz A, Needleman P (1977). Imidazole: a selective inhibitor of thromboxane synthetase. Prostaglandins 13, 611-618.

Moncada S, Gryglewski R, Bunting S, Vane JR (1976). A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance PGI_2 which prevents platelet aggregation. Prostaglandins 12, 715-737.

Moncada S, Vane JR (1978). Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. British Medical Bulletin 34, 129-135.

Nesheim B-I (1984). Induced abortion by the suction method. Acta Obstetrica et Gynaecologica Scandinavica 63, 591-595.

Newton J, Barnard G, Collins W (1977). A rapid method for measuring menstrual blood loss using automatic extraction. Contraception 16, 269-282.

Nilsson L, Rybo G (1970). Treatment of menorrhagia. American Journal of Obstetrics & Gynecology 110, 713-719.

Novak ET, Jones GS, Jones HW (1981). Abnormal uterine bleeding. In: Novak's Textbook of Gynecology. Williams & Wilkins, Baltimore. pp.777-796.

Noyes RW, Hertig AT, Rock J (1950). Dating the endometrial biopsy. Fertility and Sterility 1, 3-25.

Office of Population Censuses and Surveys (1985) Abortion Statistics. HMSO, London.

Orchard MA, Robinson C (1981). Stability of prostacyclin in human plasma and whole blood: studies on the protective effect of albumin. Thrombosis and Haemostasis (Stuttgart) 46, 645-647.

Oshima K, Matsumoto K, Tsuda T, Shibata K, Hayashi M (1978). Decreased smooth muscle side effects with 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester in Japanese monkey (Mac. Fusc.Fusc). Prostaglandins 15, 473-483.

Pakrasi PL, Dey SK (1985). Evidence for an inverse relationship between cyclooxygenase and lipoxygenase pathways in the pregnant rabbit endometrium. Prostaglandins, leukotrienes and medicine 18, 347-352.

Peek MJ, Fraser IS, Phillips CA, Resta TM, Blackwell PM, Markham R (1985). The measurement of human endometrial prostaglandin production. A comparison of two in-vitro methods. Prostaglandins 29, 3-18.

Pendergrass PB, Ream LJ, Scott JN, Agna MA (1984). Do aspirin and acetaminophen affect total menstrual loss? Gynecological and Obstetrical Investigation 18, 129-133.

Pickles VR (1957). A plain muscle stimulant in the menstruum. Nature 180, 1198-1199.

Pickles VR, Hall WJ, Best FA, Smith GN (1965). Prostaglandins in endometrium and menstrual fluid from normal and dysmenorrhoeic subjects. Journal of Obstetrics and Gynaecology of the British Commonwealth 72, 185-192.

Purandare VN, Gupta A, Rowe P, Wiqvist N, Manzano BR, Ma HK, Novikov YI, Andolsek L, Fylling P, Karim SMM, Bygdeman M, Sakamoto S (1982). Termination of early first trimester pregnancy by vaginal administration of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester. Asia-Oceana Journal of Obstetrics & Gynaecology 8, 263-268.

Rees MCP, Anderson ABM, Demers LM, Turnbull AC (1984a). Prostaglandins in menstrual fluid in menorrhagia and dysmenorrhoea. British Journal of Obstetrics & Gynaecology 91, 673-680.

Rees MCP, Anderson ABM, Demers LM, Turnbull AC (1984b). Endometrial and myometrial prostaglandin release during the menstrual cycle in relation to menstrual blood loss. Journal of Clinical Endocrinology & Metabolism 58, 813-818.

Rees MCP, di Marzo V, Tippins JR, Morris HR, Turnbull AC (1986). Leukotriene release by non pregnant human endometrium and myometrium. Proceedings of the 6th International Conference on prostaglandins and related compounds. Florence, June 1986. p. 489.

Reyniak JV, Gordon M, Stone ML, Stedis A (1975). Endometrial regeneration after voluntary abortion. Obstetrics & Gynecology 45, 203-210.

Rosen A-S, Nystedt L, Bygdeman M, Lundstrom V (1979). Acceptability of a nonsurgical method to terminate very early pregnancy in comparison to vacuum aspiration. Contraception 19, 107-117.

Rutherford RN, Mezer J (1942). Regeneration of uterine mucosa after spontaneous abortion and normal delivery. Journal of the American Medical Association. 119, 124-128.

Rybo G (1966) Clinical and experimental studies on menstrual blood loss. Acta Obstetrica et Gynaecologica Scandinavica 45 (Suppl) 1-23.

Saksena SK, Harper MJK (1974). Prostaglandin - mediated action of intrauterine devices: F prostaglandins in the uterine horns of pregnant rabbits with unilateral intrauterine devices. Fertility & Sterility 25, 121-126.

Schatz F, Markiewicz L, Barg, P, Gurpide E (1985). In vitro effects of ovarian steroids on prostaglandin $F_{2\alpha}$ output by human endometrium and endometrial epithelial cells. Journal of Clinical Endocrinology & Metabolism 61, 361-367.

Scommegna A, Ilekis J, Rao R, Dmowski WP, Rezai P, Auletta FJ (1978). Endometrial PGF content in women wearing non-medicated or progestin-releasing IUCD's. Fertility & Sterility 29, 500-504.

Shapiro SS, Haning RV (1983). Ibuprofen inhibits prostaglandin $F_{2\alpha}$ during menstruation. Current therapeutic research 33, 380-384.

Shaw ST, Macauley LK, Aznar R, Gonzalez-Angulo A, Roy S (1981). Effects of a progesterone-releasing intrauterine contraceptive device on endometrial blood vessels: a morphometric study. American Journal of Obstetrics & Gynecology 141, 821-827.

Sidhu MS, Kent DR (1984). Effects of prostaglandin E_2 analogue suppository on blood loss in suction abortion. Obstetrics & Gynecology 64, 128-130.

Simpson JY (1863) Clinical lectures of the diseases of women. Blanchard & Lea, Philadelphia.

Siegel S (1956). Nonparametric statistics for the behavioural sciences. McGraw Hill.

Smith JB, Willis AL (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biology 231, 235-237.

Smith SK (1982). Physiological and pharmacological aspects of prostaglandins in the female reproductive tract. M.D. Thesis, University of London.

Smith SK, Abel MH, Kelly RW, Baird DT (1981a). Prostaglandin synthesis in the endometrium of women with ovular dysfunctional

uterine bleeding. British Journal of Obstetrics & Gynaecology 88, 434-442.

Smith SK, Abel MH, Kelly RW, Baird DT (1981b). A role for prostacyclin (PGI_2) in excessive menstrual bleeding. Lancet, i., 522-524.

Smith SK, Abel MH, Kelly RW, Baird DT (1982). The synthesis of prostaglandins from persistent proliferative endometrium. Journal of Clinical Endocrinology & Metabolism 55, 284-89.

Smith SK, Baird DT (1980). The use of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester (ONO 802) vaginal suppositories for the termination of early pregnancy. A comparative study. British Journal of Obstetrics & Gynaecology 87, 712-717.

Soderstrom RM (1979). Menstrual regulation technology. In: Pregnancy termination-procedures, safety and new developments. Editors: Zatuchni GI, Sciarra JJ, Speidal JJ. Harper & Rowe. pp. 60-68.

Spilman CH, Beuring DC, Forbes AD, Roseman TJ, Bennet RM, Biermacher JJ, Tuttle ME (1984). Menses induction in rhesus monkeys using a controlled-release vaginal delivery system containing (15 S) 15 methyl prostaglandin $F_{2\alpha}$methyl ester. Fertility & Sterility 42, 638-643.

Stringer J, Anderson M, Beard RW, Fairweather DVI, Steele SJ (1975). Very early termination of pregnancy (menstrual extraction). British Medical Journal 3, 7-9.

Swahn ML, Cekan S, Wang G, Lundstrom V, Bygdeman M (1985a). Pharmacokinetic and clinical studies of RU486 for fertility regulation. In: The antiprogestin steroid RU486 and human fertility control. Eds: Baulieu E-E, Segal SJ, Plenum Press, New York. pp 249-258.

Swahn ML, Cekan S, Wang G, Lundstrom V, Bygdeman M (1985b). Clinical experience with antiprogestins. ICMR/WHO Scientific Meeting: Bangalore, India.

Takagi S, Sakata H, Yoshida T, Den K, Fujii TK, Amemiya H, Tomita M (1978). Termination of early pregnancy by ONO-802 suppositories (16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester). Prostaglandins 15, 913-919.

Tan KC, Goh VHH, Karim SMM, Ratnam, SS, Koltegoda SR (1984). Maternal plasma estradiol and progesterone levels during therapeutic abortion induced by 16,16 dimethyl PGE_2 p-benzaldehyde semicarbazone ester. Prostaglandins, leukotrienes and medicine 14, 215-224.

Tauber PF, Kloppel A, Goodpasture JC, Burns J, Ludwig H, Zaneveld LJD (1981). Reduced menstrual blood loss by release of an antifibrinolytic agent from IUCDs. American Journal of Obstetrics & Gynecology 140, 322-328.

Troughbough G, Guderian AM, Erickson RR, Tillson SA, Leong P, Swisher DA, Pharriss BB (1978). Effect of exogenous intrauterine progesterone on amount and $PGF_{2\alpha}$ content of menstrual blood in

dysmenorrhoeic women. Journal of Reproductive Medicine 21, 153-158.

Tsang BK, Ooi TC (1982). Prostaglandin secretion by human endometrium in vitro. American Journal of Obstetrics & Gynecology 142, 626-633.

Tseng L, Lazella J, Mann WJ, Chumas J (1982). Oestrogen synthesis in normal and malignant human endometrium. Journal of Clinical Endocrinology & Metabolism 55, 1029-1031.

Tyler HM, Saxton CAPD, Parry MJ (1981). Administration to man of UK-37, 248-01, a selective inhibitor of thromboxane synthetase. Lancet i, 629-632.

Vaitukaitis JL, Braunstein GD, Ross GT (1972). A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of luteinising hormone. American Journal of Obstetrics & Gynecology 113, 751-758.

Van Dijk JG, Frohlich M, Brand EC, Van Hall EV (1979). The treatment of unexplained infertility with danazol. Fertility and Sterility 31, 481-485.

Van Look PFA, Lothian H, Hunter WM, Michie EA, Baird DT (1977). Hypothalamic-pituitary-ovarian function in perimenopausal women. Clinical Endocrinology 7, 13-31.

Vane JR (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology 231, 232-235.

Vijayakamur R (1980). The role of utero-ovarian tissue prostaglandins in the human menstrual cycle. PhD Thesis. Monash University, Melbourne.

Von Euler US (1934). Zur Kenntis der pharmakologischen wirkungen von nativsekreten und extracten mannlicher accessorischer geschlechtsdrusen. Archiv fur experimentelle pathologie und pharmakologie, 175, 78-84.

Webster MA, Pattison NS, Phipps SL, Gillmer MDG (1985a). Myometrial activity in first trimester human pregnancy after epostane therapy. Effect of intravenous oxytocin. British Journal of Obstetrics & Gynaecology 92, 957-962.

Webster MA, Phipps SL, Gillmer MDG (1985b). Interruption of first trimester human pregnancy following epostane therapy. Effect of prostaglandin E_2 pessaries. British Journal of Obstetrics & Gynaecology 92, 963-968.

West NB, Brenner RM (1985). Progesterone-mediated suppression of estradiol receptors in cynomolgus macaque cervix, endometrium and oviduct during sequential estradiol-progesterone treatment. Journal of steroid biochemistry 22, 29-37.

Williams JW (1931) Regeneration of the uterine mucosa after delivery, with especial reference to the placental site. American Journal of Obstetrics & Gynecology 22, 664-696 and 793-796.

Willman EA, Collins WD, Clayton SG (1976). Studies on the

involvement of prostaglandins in uterine symptomatology and pathology. British Journal of Obstetrics & Gynaecology 83, 337-341.

Wilson T, Liggins GC, Aimer GP, Watkins EJ (1986). The effect of progesterone on the release of arachidonic acid from human endometrial cells stimulated by histamine. Prostaglandins 31, 343-360.

Wiqvist N, Bygdeman M, Kirton K (1971). Non-steroidal infertility agents in the female. In: Control of Human Fertility. Nobel Symposium 15. Editors: Diczfalusy E, Borell B, 137-146.

Ylikorkala O, Viinikka L (1983). Comparison between antifibrinolytic and antiprostaglandin treatment in the reduction of increased menstrual blood loss in women with intrauterine contraceptive devices. British Journal of Obstetrics & Gynaecology 90, 78-83.

Zahradnik HP, Stengele E, Kraut E, Scharpf G, Breckwoldt M (1978). Neue aspekte zur pathogenese und therapie der dysmenorrhoe. Deutsch Medizinische Wochenschrift (Stuttgart) 103, 1270-1273.

APPENDIX 1: ABBREVIATIONS

The following abbreviations have been employed.

FSH	Follicle stimulating hormone
hCG	Human chorionic gonadotrophin
IPT	Immunological pregnancy test
LH	Luteinising hormone
MBL	Menstrual blood loss
MOX	methyl oxime
PG	Prostaglandin
SEM	Standard error of mean
TX	Thromboxane
$t_{\frac{1}{2}}$	half life

APPENDIX 2: STATISTICAL METHODS

Non parametric tests have been employed (Siegel, 1956). The differences between paired and independent samples have been assessed using the Wilcoxon signed rank and Wilcoxon rank sum tests respectively. Analysis of frequency tables has been performed using the χ^2 test and the Fisher exact probability test, and bivariate data have been analysed using Spearman's rank correlation.

APPENDIX 3: PUBLISHED ARTICLES

The following have been published, based on the text of the thesis.

1. Cameron IT, Kelly RW, Baird DT (1985). Prostaglandins in the human uterus: an interaction between endometrium and myometrium. Prostaglandins, Leukotrienes and Medicine, 17, 329-335.

2. Cameron IT, Baird DT (1986). A controlled release form of 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester for early abortion. Contraception, 33, 121-125.

3. Cameron IT, Michie AF, Baird DT (1986). Therapeutic abortion in early pregnancy with antiprogestogen RU486 alone or in combination with prostaglandin analogue (Cervagem) Contraception, 34, 459-468.

4. Cameron IT, Leask R, Kelly RW, Baird DT (1987). Endometrial prostaglandins in women with abnormal menstrual bleeding. Prostaglandins, Leukotrienes & Medicine (in press).

5. Cameron IT, Leask R, Kelly RW, Baird DT (1987). The effects of Danazol, Mefenamic Acid, Norethisterone and a progesterone-impregnated coil, on endometrial prostaglandin concentrations in women with menorrhagia. Prostaglandins (in press).

6. Cameron IT, Baird DT (1987). Early pregnancy termination: A comparison between vacuum aspiration and medical abortion using prostaglandin (16,16 dimethyl-trans-Δ_2 -PGE_1 methyl ester) or the antiprogestogen RU 486. British Journal of Obstetrics & Gynaecology (in press).

Additional related articles include:

1. Cameron IT, Kelly RW, Baird DT (1985). Dysfunctional uterine bleeding: a role for the prostaglandins (Blair Bell Research Society). British Journal of Obstetrics and Gynaecology, 93, 91.

2. Kelly RW, Healy DL, Cameron MJ, Cameron IT, Baird DT (1985). RU486 stimulates $PGF_{2\alpha}$ production in isolated endometrial cells in short term culture. In: The antiprogestin steroid RU486 and human fertility control. Editors: Baulieu E-E, Segal SJ, Plenum Press, N.Y. pp. 259-262.

3. Baird DT, Cameron IT (1985). Menstrual induction: surgery versus prostaglandins. In: Abortion: medical progress and social implications. (CIBA Foundation Symposium 115). John Wiley & Sons Ltd, U.K. pp 178-191.

4. Kelly RW, Healy DL, Cameron MJ, Cameron IT, Baird DT (1986). The stimulation of prostaglandin production by two antiprogesterone steroids in human endometrial cells. Journal of Clinical Endocrinology and Metabolism, 62, 1116-1123.

5. Healy DL, Cameron IT (1987). Clinical physiology of the endometrium. In: Reproductive physiology in clinical practice. Editor: Riddick D., Thieme Stratton, N.Y. pp. 51-70.

Prostaglandins Leukotrienes and Medicine 17: 329-335, 1985

PROSTAGLANDINS IN THE HUMAN UTERUS: AN INTERACTION BETWEEN ENDOMETRIUM AND MYOMETRIUM

[1]I. T. Cameron, [2]R.W. Kelly & [1]D.T. Baird

[1]Dept. of Obstetrics & Gynaecology, University of Edinburgh and [2]MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh. EH3 9EW. Scotland. U.K. (reprint requests to ITC).

ABSTRACT

The interaction between the prostaglandin production of the human endometrium and myometrium has been further investigated using intact pieces of endometrium in homogenates of myometrium. There was an increase in the production of 6 oxo $PGF_1\alpha$ when these tissues were incubated together, whereas there was no change in the production of PGE, $PGF_2\alpha$ or 13, 14 dihydro 15 - keto $PGF_2\alpha$. This endometrial - myometrial interaction could have important implications in the pathogenesis of menorrhagia.

INTRODUCTION

A role for the prostaglandins (PG) in local uterine pathophysiology has been suggested for some time, and since the discovery of an acidic lipid in the menstruum with oxytocic properties (1), later found to be PGE_2 and $PGF_2\alpha$ (2), these substances have been implicated in the aetiology of various disorders of menstruation.

An increase in the concentration of both $PGF_2\alpha$ and PGE_2 has been measured in the menstrual fluid of patients with dysmenorrhoea (3), whereas excessive menstrual bleeding appears to be associated with an imbalance in uterine PG production (4,5). Endometrium from women with ovulatory dysfunctional uterine bleeding synthesises more PGE_2 than that of normal women, and in individuals with anovulatory dysfunctional bleeding, excessive menstrual loss is associated with a relative deficiency in $PGF_2\alpha$ synthesis (6). There is an inverse relationship between the ratio of $PGF_2\alpha$:PGE_2 and the amount of blood lost, and it may be therefore that menstrual loss is determined by the relative synthesis of prostaglandins with mainly vasoconstrictor properties on the one hand ($PGF_2\alpha$) as opposed to those with vasodilatory properties (PGI_2,PGE_2) on the other (7).

Within the uterus the major prostaglandin output is derived from the endometrium, with the prostaglandins E_2 and $F_{2\alpha}$ predominating, although small amounts of prostacyclin, measured as 6 oxo $PGF_{1\alpha}$, are found (8). This contrasts with the situation in the myometrium, where 6 oxo $PGF_{1\alpha}$ is the major product of arachidonic acid metabolism in both animals (9) and man (10). An interaction between the PGs from the two uterine tissues has been suggested by Smith et al (11), who demonstrated that the endometrium from women with menorrhagia could enhance the myometrial production of prostacyclin, and more recently these observations have been confirmed (8). Each of these studies has employed broken cell preparations, both with and without incubation with ^{14}C - labelled arachidonic acid respectively.

The present work has further investigated the interaction between homologous endometrium and myometrium, using intact pieces of endometrium. In addition to assessing the change in the synthesis of PGE_2, $PGF_{2\alpha}$ and 6 oxo $PGF_{1\alpha}$, the effect of this combined tissue incubation on the major metabolite of $PGF_{2\alpha}$, 13, 14 dihydro 15 - keto $PGF_{2\alpha}$ ($PGFM_2$), has also been observed.

METHODS

Tissue Collection and Prostaglandin Measurement

6 women suffering from benign uterine disease, each with a subjective complaint of menorrhagia, were admitted for abdominal hysterectomy in the luteal phase of the cycle. At operation, samples of both endometrium and myometrium were placed in ice - cold modified 199 medium (Flow Laboratories, U.K.), and transported to the laboratory. After blotting and weighing both types of tissue, a myometrial homogenate was prepared in 199 medium (using a Polytron homogeniser), at a final concentration of 10 mg/ml. Thereafter, small pieces of endometrium (5.2$\pm$ 0.3 mg(mean $\pm$ S.E.M) n = 48) were placed in either 1 ml of 199 medium or 1 ml of the myometrial homogenate, and these samples were incubated at 37°C in a water bath for 1 hour , with gentle shaking. 1 ml samples of the myometrial homogenate alone, and control samples of 199 medium were also processed in similar fashion. Following the incubation, the pieces of endometrium were removed, and the media were stored at -20°C until assayed.

Prostaglandin concentrations were measured using standard radioimmunoassay techniques (12). Antibodies raised against PGE_2 and $PGF_{2\alpha}$ (Dr J. Hennam, Kings College, London), 6 oxo $PGF_{1\alpha}$ (Dr J.A. Salmon, Wellcome Research Laboratories, Beckenham), and $PGFM_2$ (Upjohn Ltd), were employed - and as the antibody against PGE_2 cross-reacted significantly with PGE_1, the value for the concentration of PGE presented includes prostaglandin from both the 1 and 2 series. Inter and intra assay coefficients of variation, taken over 10 sequential assays and in duplicate 10 times within the same assay, were 12.2% and 14.4% for $PGF_{2\alpha}$, 10.1% and 11.2% for PGE, 11.1% and 14.5% for 6 oxo $PGF_{1\alpha}$, and 11.0% and 13.7% for $PGFM_2$.

Endometrial Dating

In all experiments, a portion of endometrium was placed in formol saline for histological dating (13). The occurrence of ovulation was indicated by the presence of secretory endometrium, along with a serum progesterone concentration greater than 9 nmol/L.

Statistical Analysis

Non parametric tests were used (14), the significance between paired samples being assessed by the Wilcoxon signed - rank test.

RESULTS

The characteristics of the 6 patients are seen in Table 1. All were in the secretory phase of the cycle, and all showed histological evidence of luteal function. Each of the women complained of heavy periods, with associated dysmenorrhoea in two cases, however their menstrual loss was not assessed objectively.

	AGE (yrs)	PARITY	CYCLE	DAY
1	46	3+0	4/28	22
2	43	1+0	8/25	26
3	46	4+2	8/26	25
4	35	4+1	11/24	24
5	40	2+0	7/23	22
6	40	4+0	6/21	17

Table 1 Patient characteristics.

Endometrial prostaglandin production, both alone and in combination with myometrium, is presented in Figure 1. After correcting for the production of prostaglandin by the myometrial homogenate alone, the value for the combined incubate is expressed in pg/mg endometrium per hour. As can be seen, there was an increase in the production of 6 oxo $PGF_{1\alpha}$ by the endometrium when incubated with myometrium, however, there was no significant change in the production of either PGE, $PGF_{2\alpha}$, or the metabolite $PGFM_2$.

DISCUSSION

It has previously been shown that endometrial homogenates from women with menorrhagia have a greater capacity than normal endometrium to generate 6 oxo $PGF_{1\alpha}$ when incubated in combination with control homogenates of myometrium (11). This increased 6 oxo $PGF_{1\alpha}$ production

was found by incubating the tissues with exogenous precursor in the form of ^{14}C labelled arachidonic acid. Dimov et al, (15) have recently questioned the validity of such incubation techniques, suggesting that only qualitative conclusions should be drawn. However, Kelly et al (8), again working with both endometrial and myometrial homogenates, and with the endometrium itself acting as the source of endoperoxide precursor, have confirmed the increase in 6 oxo $PGF_1\alpha$ seen on combined incubation. Furthermore, this group has shown that the variation in 6 oxo $PGF_1\alpha$ production is a characteristic of the endometrium itself, rather than the myometrium.

The present work further supports the hypothesis that when these two uterine tisssues are combined, the intact endometrium can supply precursor endoperoxide to the myometrium, where it is converted primarily to prostacyclin and thence to 6 oxo $PGF_1\alpha$. That it has not been necessary to prepare an endometrial homogenate to demonstrate this increase in 6 oxo $PGF_1\alpha$ production would suggest that the intact endometrium could release precursor endoperoxide in vitro. There was no change in the production of PGE, $PGF_2\alpha$ or $PGFM_2$ on combined incubation, in agreement with previous observations (11), although Abel and Kelly (10) have suggested that a decrease in the production of PGE and $PGF_2\alpha$ may result from the diversion of endoperoxide towards prostacyclin synthesis.

The variable degree by which the 6 oxo $PGF_1\alpha$ concentration increased in the present experiments (by a factor of 1.3 to 6.7 times), might have been related to the level of menstrual blood loss. However, further studies recruiting women with objectively assessed periods would be necessary to test this, for it is evident that almost 50% of women presenting with a subjective complaint of menorrhagia will have a measured monthly blood loss within normal limits (16).

In conclusion, the presented work illustrates that an interaction between endometrial and myometrial prostaglandin production is possible, and that endoperoxide precursor can be released from intact endometrium. Although such in vitro data do not necessarily prove that this mechanism exists in vivo, it is interesting to consider its possible consequences. Kelly et al, (8) have recently suggested that there might be an increased availability of arachidonic acid in the endometrium of women suffering from menorrhagia. In the event of the resultant increase in precursor endoperoxide availability, not only might the synthetic pathway towards $PGF_2\alpha$ become saturated, leading to a diversion of endoperoxide towards PGE_2 non-enzymatically (and thus causing an increase in the PGE_2:$PGF_2\alpha$ ratio), but also more PGI_2 could be formed, both in the endometrium, and, in addition, in the large muscle mass of the myometrium. This increase in prostacyclin production could have important implications in the pathogenesis of menorrhagia.

ACKNOWLEDGEMENT

We thank the Consultant staff of the Royal Infirmary, Edinburgh, for allowing access to tissues at operation, and Dr J.A. Salmon, Wellcome Research Laboratories, for the generous gift of the 6 oxo $PGF_1\alpha$

antiserum. Thanks are also due to Miss R. Leask for invaluable technical assistance, and to Mr T. McFetters and Miss M. Seenan, for help with illustrations and typing respectively. Histological dating, performed by Mr L. McKenzie, is gratefully acknowledged. ITC has been in receipt of a Birthright Research Grant (Royal College of Obstetricians and Gynaecologists).

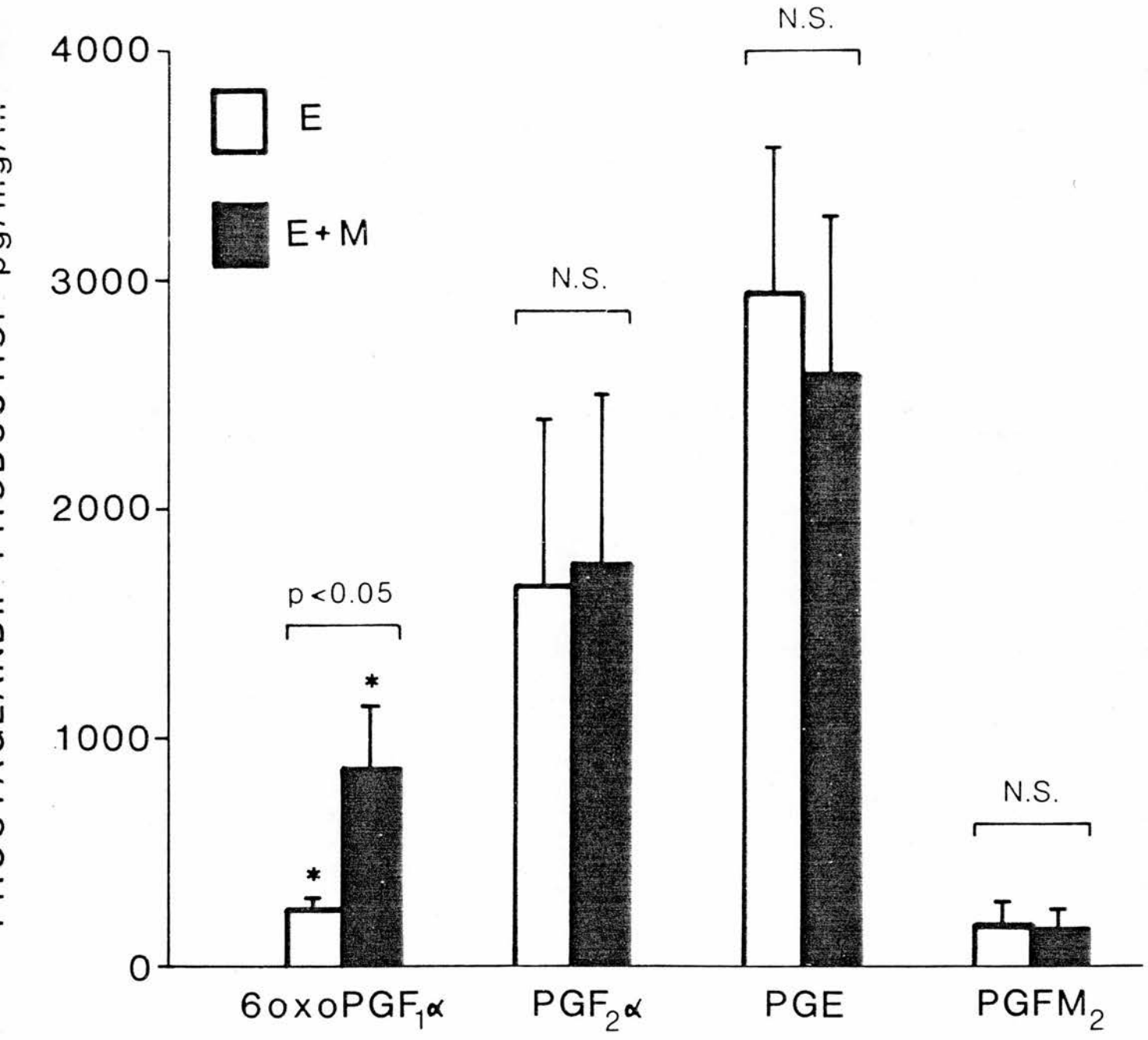


Figure 1 Combined endometrial - myometrial incubations: prostaglandin production in pg/mg/hr (mean ± standard error of the mean - n = 6 in each case). E - endometrium alone: E + M - endometrium plus myometrium.

Pickles VR. A plain muscle stimulant in the menstruum. Nature 180: 1198, 1957.

Pickles VR, Hall WJ, Best FA, Smith GN. Prostaglandins in endometrium and menstrual fluid from normal and dysmenorrhoeic subjects. J. Obstet. Gynaecol. Brit. Commw. 72: 185, 1965.

Lumsden MA, Kelly RW, Baird DT. Primary dysmenorrhoea: the importance of both prostaglandins E_2 and $F_{2\alpha}$. Brit. J. Obstet. Gynaecol. 90: 1135, 1983.

Smith SK, Abel MH, Kelly RW, Baird DT. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. Brit. J. Obstet. Gynaecol. 88: 434, 1981.

Willman EA, Collins WD, Clayton SG. Studies on the involvement of prostaglandins in uterine symptomatology and pathology. Br. J. Obstet. Gynaecol. 83: 337, 1976.

Smith SK, Abel MH, Kelly RW, Baird DT. The synthesis of prostaglandins from persistent proliferative endometrium. J. Clin. Endocrinol. Metab. 55: 284, 1982.

Baird DT, Abel MH, Kelly RW, Smith SK. Endocrinology of dysfunctional uterine bleeding: the role of endometrial prostaglandins. In: Endocrinology of Human Infertility: New Aspects. Serono clinical colliquia on Reproduction. 2. Eds: Crosignani, P.G. & Rubin, B.L. Academic Press. London. p 399, 1981.

Kelly RW, Lumsden MA, Abel MH, Baird DT. The relationship between menstrual blood loss and prostaglandin production in the human: evidence for increased availability of arachidonic acid in women suffering from menorrhagia. Prostaglandins, Leuk. & Med. 1984 (in press).

Jones RL, Poyser NL, Wilson NH. Production of 6 oxo $PGF_{1\alpha}$ by rat, guinea-pig and sheep uteri in vitro. Brit. J. Pharmacol. 59: 436, 1977.

Abel MH, Kelly RW. Differential production of prostaglandins within the human uterus. Prostaglandins. 18: 821, 1979.

Smith SK, Abel MH, Kelly RW, Baird DT. A role for prostacyclin (PGI_2) in excessive menstrual bleeding. Lancet, i: 522, 1981.

Abel MH, Smith SK, Baird DT. Suppression of concentration of endometrial prostaglandin in early intra-uterine and ectopic pregnancy in women. J. Endocr. 85: 379, 1980.

Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil. Steril. 1: 3, 1950.

14 Siegel S. The Wilcoxon matched - pairs signed - ranks test. In: Nonparametric statistics for the behavioral sciences. McGraw Hill. p 75, 1956

15 Dimov V, Christensen NJ, Green K. Analysis of prostaglandins formed from endogenous and exogenous arachidonic acid in homogenates of human reproductive tissues. Biochim. et. Biophys. Acta. 754: 38, 1983.

16 Fraser IS, Pearse C, Shearman RP, Elliott PM, McIlveen J, Markham R. Efficacy of mefenamic acid in patients with a complaint of menorrhagia. Obstet. Gynecol. 58: 543, 1981.

A CONTROLLED RELEASE FORM OF 16,16—DIMETHYL-TRANS-Δ_2-PGE METHYL ESTER FOR EARLY ABORTION

I.T. CAMERON, Lecturer, and D.T. BAIRD, Professor,
Department of Obstetrics and Gynaecology, University of Edinburgh,
Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh. EH3 9EW

ABSTRACT

The termination of early pregnancy (<56 days amenorrhoea) has been investigated using 16,16—dimethyl-trans-Δ_2-PGE, methyl ester in a controlled release preparation. The onset of crampy abdominal pain was seen after 270 ± 39 minutes and bleeding occurred after 603 ± 95 minutes. Two (15%) patients required no pain relief during treatment, however 5 (38%) requested oral analgesia, and in 6 (46%) individuals the pain was severe enough to warrant parenteral opiates. The overall success rate for complete abortion was 85%. No serious adverse effects were seen, but vomiting occurred in 2 (15%) women, and diarrhoea in 3 (23%). Although the use of this prostaglandin analogue in slow release form provides an effective treatment method for early abortion using a reduced total dose of prostaglandin, the acceptability of the drug as an agent for menstrual induction continues to be limited by the occurrence of troublesome gastro-intestinal side effects.

INTRODUCTION

Since the introduction of the 1967 Abortion Act, the annual number of therapeutic abortions has increased steadily, and in 1982 a total of 163,045 pregnancies were terminated in England and Wales (1). In Scotland, after an initial upward trend, the annual number of such abortions performed has reached a plateau, and in 1983,8,419 cases were reported, 89% of which were at a gestation of 13 weeks or less.

Vacuum aspiration of the uterine cavity under general anaesthesia continues to be the most widely accepted method for pregnancy termination in the first trimester. However, such procedures are not without risk, and the recognition that the complication rate associated with therapeutic abortion is related to both the gestational age and the anaesthetic and surgical techniques employed, has led to the development of a medical approach to early abortion.

Initially the naturally occurring prostaglandins (PG) of the E and F series were used (2), and subsequently a number of analogues have been synthesised, with increased stability and more specific uterine activity (3,4,5), but gastro-intestinal associated side effects have continued to be a major problem, occurring in up to 40% of cases (6).

Submitted for publication January 21, 1986
Accepted for publication January 28, 1986

Although a lower dose of prostaglandin can be used to decrease the incidence and severity of these side effects, this may lead to a reduced success rate for abortion itself. An alternative solution would be to administer the prostaglandin in a controlled release form, thereby lessening the "bolus effect" each time an individual dose of drug is given.

The present work has investigated the use of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester incorporated into such a controlled release device, for the interception of early pregnancy (<56 days amenorrhoea).

PATIENTS AND METHODS

Thirteen women with pregnancies of less than 56 days gestation were recruited for study. Gestational age was assessed by menstrual history and clinical examination, with the addition of pelvic ultrasound where necessary. In all cases, pregnancy was confirmed with an immunological pregnancy test (7). Patients with evidence of abnormal pregnancy or spontaneous abortion were excluded from study, as were women with medical complications such as cardiovascular or pulmonary disease, allergy, or epilepsy. Pregnancy termination was indicated under the 1967 Abortion Act: local ethical committee approval was granted for the project, and written informed consent was obtained from each woman prior to proceeding with treatment.

On admission to hospital, a device containing 3 mg of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester incorporated into a strip of polyethylene-oxide based hydrogel (measuring 1.3 x 10 x 30 mm) was placed in the posterior fornix of the vagina. After 1 hour, the women were allowed to get up, and they remained in hospital for 24 hours. Prophylactic medications were not prescribed, however analgesics (paracetamol, 1 gm; dihydrocodeine, 30 mg; or pethidine 100 mg), antiemetics (cyclizine, 50 mg) or other preparations, such as antidiarrhoeal agents, were administered on request. No dietary restriction was enforced during treatment.

The devices were removed prior to discharge from hospital. Patients were given appropriate contraceptive advice, and arrangements were made for follow-up at 1 week, 2 weeks, 4 weeks and fortnightly thereafter, until the onset of the next period. The abortion was considered successful at the 2-week visit if there was minimal vaginal bleeding, the cervix was closed, the uterus was normal in size, and the pregnancy test indicated that the concentration of human chorionic gonadotrophin was falling.

RESULTS

The 13 women, whose average age was 26.0 ± 1.9 years (mean ± SEM), were at a mean gestation of 51.1 ± 1.2 days. Four (31%) individuals were parous, and one had undergone previous pregnancy termination in the second trimester.

Crampy abdominal pain was seen after 270 ± 39 minutes, and the onset of bleeding occurred after 603 ± 95 minutes. The degree of blood loss was not assessed objectively, but in no case was the subjective loss so great as to warrant either blood transfusion or emergency uterine evacuation.

Only 2 patients required no pain relief during treatment. Five requested oral analgesia, and 6 considered the pain severe enough to require parenteral opiates. Two women suffered from vomiting, and diarrhoea occurred in 3.

The successful induction of bleeding followed by abortion occurred in 11 (85%) women without the need for surgical intervention. The mean duration of bleeding in these individuals was 10.9 $\pm$ 1.7 days, and in 8 (73%) cases, mild period-like pain was experienced during the first week of follow-up.

The remaining two women underwent uterine evacuation under general anaesthesia. One patient was admitted on the fourth day post-treatment with abdominal pain and vaginal discharge; a clinical diagnosis of retained products of conception was made, and she proceeded to surgical evacuation under antibiotic cover. The second woman underwent uterine evacuation and diagnostic laparoscopy on the 24th day following treatment because of continuing vaginal bleeding, tenderness in the right fornix, and a persisting positive pregnancy test. Operation revealed a luteal cyst on the right ovary, and curettage confirmed the presence of chorionic villi in the uterus.

The amount of drug administered to each patient was measured by assaying the residual compound in the device, using high pressure liquid chromatography. The median dose received was 1.65 mg (range 1.16-1.86 mg), representing a percentage release of 53% (range 39%-62%). There was no relationship between the success rate or the incidence of side effects, and the dose of drug administered.

DISCUSSION

Although menstrual induction has been performed using a variety of synthetic prostaglandin analogues, with success rates of up to 90-95%, side effects, particularly associated with the gastro-intestinal tract, have limited the acceptability of these drugs as therapeutic agents. The incidence of vomiting and diarrhoea may be reduced by routine premedication with antiemetic or anti-diarrhoeal agents (8,9). Alternatively, the use of a lower dose of the prostaglandin itself would improve these dose-related side effects, but this may lead to a reduced success rate for abortion itself. However, the administration of the prostaglandin in a local controlled release form will lessen the "bolus effect" each time an individual dose of drug is given, and may maintain an acceptable success rate with a reduced total drug dosage.

The present work has assessed the use of a device releasing 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester in controlled release fashion. This prostaglandin analogue has been widely employed to induce therapeutic abortion in both the first and second trimesters of pregnancy in the form of 1 mg vaginal pessaries (10,11,12) and more recently it has been synthesised in a slow release device made of a 3-layered water soluble polymer (13). In the latter study, abortion was achieved in 21 (84%) late second trimester pregnancies within 18 hours of treatment with devices containing 1.5-2.0 mg of drug, but vomiting and diarrhoea were seen in 9 (35%) and 3 (12%) cases, respectively.

Here the drug has been incorporated into a strip of polyethylene oxide-based hydrogel in which cross-linking is induced by the use of an aliphatic difunctional isocyanate and an aliphatic triol. On exposure to body fluids, the polymer swells to about three times its dry volume, at a rate which can be predetermined by the composition of the matrix. The drug then diffuses out of the matrix at a zero order rate, over a time period dependent upon the thickness of the device, the degree of crystallinity of the polymer, and the actual prostaglandin used. All the drug is not released within 24 hours, however measurement of the residual prostaglandin in the devices themselves indicated a reproducible release pattern.

The success rate of 85% compares well with that of other studies using this prostaglandin in pessary form, however the incidence of vomiting and diarrhoea is not significantly different from that previously cited by Smith and Baird (11) in 1980 ($p>0.05$). Additionally, in a further 30 women treated with 16,16 dimethyl-trans-Δ_2-PGE_1 methyl ester in pessary form, the incidence of vomiting (23%) and diarrhoea (33%) was similar to that seen using the slow release device (Cameron & Baird, unpublished data).

Although safe and effective, the failure rate of 5-15%, resulting in the need for stringent follow—up arrangements, does limit the use of such medical agents for menstrual induction. In addition, at vacuum aspiration it is possible to inspect the products of conception to confirm the presence of an intra-uterine gestation. This assumes more importance especially in cases of very early abortion with minimal uterine enlargement, and in situations where associated predisposing factors for ectopic implantation exist.

In conclusion, the use of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester in this slow release form provides an effective, safe treatment method for early abortion. However, although a reduced dose of prostaglandin is employed, there is no consistent improvement in the incidence of gastro-intestinal associated side effects, compared with other studies using the same prostaglandin in pessary form.

ACKNOWLEDGEMENTS

We are grateful to Sisters D. Will, J. McKinnon and staff for their assistance with patient care, and to Sister A. Michie for help with follow—up and data collection. Thanks are also due to Miss A. Maloney for typing the manuscript. 16,16—Dimethyl-trans-Δ_2-PGE_1 methyl ester was kindly supplied by May & Baker Ltd., in a slow release device developed in collaboration with Professor Neil Graham and the late Dr. Mostyn P. Embrey. The prostaglandin remaining in the devices was assayed by Mr. M.C. Hart, Mr. P.H. Leigh and Dr. D.J. Mills, of the Analytical Development Laboratories, May & Baker, Ltd.

REFERENCES

1. Office of Population Censuses and Surveys. Abortion statistics. HMSO, London (1982)
2. Mocsary, P. & Csapo, A.I. Menstrual induction with $PGF_{2\alpha}$ and PGE_2. Prostaglandins 10: 545-547 (1975)
3. Mackenzie, I.Z., Davies, A.J., Embrey, M.P. & Guillebaud, J. Very early abortion by prostaglandins. Lancet i: 1223-1226 (1978)
4. Bygdeman, M., Bremme, K., Christensen, N., Lundström, V. & Gréen, K. A comparison of two stable prostaglandin E analogues for termination of early pregnancy and for cervical dilatation. Contraception 22: 471-483 (1980)
5. Csapo, A.I., Peskin, E.G., Pulkkinen, M., Laajoki, V., Kivikoski, A., Lampe, L., Godeny, S., Szeverenyi, M., Herczeg, J., Resch, B. & Bacos, L. "Menstrual induction " with sulprostone. Prostaglandins 24: 657-665 (1982)
6. Bygdeman, M. & Gréen, K. Menstrual regulation: Medical techniques. In: Pregnancy Termination - Procedures, Safety and New Developments (G.I. Zatuchni, J.J. Sciarra & J.J. Speidel, Editors). Harper & Row, 1979, pp 69-77
7. Hobson, B.M. Pregnancy diagnosis using the pregnosticon haemagglutination inhibition test. Brit. J. Obstet. Gynaecol. 75: 718-723 (1968)
8. Bygdeman, M., Gréen, K., Lundström, V., Ramadan, M., Fokiou, S. & Bergström, S. Induction of abortion by vaginal administration of 15(S)15-methyl prostaglandin $F_{2\alpha}$ methyl ester. A comparison of two delivery systems. Prostaglandins 12: Suppl. 27-51 (1976)
9. Mandelin, M. & Kajanoja, P. Induction of second trimester abortion: comparison between vaginal 15-methyl-$PGF_{2\alpha}$ methyl ester and intra-amniotic $PGF_{2\alpha}$. Prostaglandins 16: 995-1001 (1978)
10. Takagi, S., Sakata, H., Yoshida, T., Den, K., Fujii, T.K., Amemiya, H. & Tomita, M. Termination of early pregnancy by ONO-802 suppositories (16,16—dimethyl-trans-Δ_2-PGE_1 methyl ester). Prostaglandins 15: 913-919 (1978)
11. Smith, S.K. & Baird, D.T. The use of 16,16—dimethyl-trans-Δ_2-PGE_1 methyl ester (ONO 802) vaginal suppositories for the termination of early pregnancy. A comparitive study. Brit. J. Obstet. Gynaecol. 87: 712-717 (1980)
12. Cameron, I.T. & Baird, D.T. The use of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester (Gemeprost) vaginal pessaries for the termination of pregnancy in the early second trimester. A comparison with extra-amniotic prostaglandin E_2. Brit. J. Obstet. Gynaecol. 91: 1136-1140 (1984)
13. Bygdeman, M., Christensen, N.J., Dimov, V. & Gréen, K. Termination of pregnancy by a slow release device containing 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester. Asia-Oceania J. Obstet. Gynaecol. 10: 359-365 (1984)

THERAPEUTIC ABORTION IN EARLY PREGNANCY WITH ANTIPROGESTOGEN RU486 ALONE OR IN COMBINATION WITH PROSTAGLANDIN ANALOGUE (Gemeprost)

I. T. CAMERON*, A. F. MICHIE and D. T. BAIRD

Department of Obstetrics and Gynaecology
University of Edinburgh
Centre for Reproductive Biology
37 Chalmers Street
Edinburgh, U.K. EH3 9EW

Abstract

Abortion was attempted in 39 women in early pregnancy (<56 days amenorrhea) with the progesterone antagonist RU486 alone (150 mg per day for 4 days) or in combination with a PG analogue, 16,16-dimethyl-trans-Δ_2-PGE_1 (Gemeprost) in the form of a 1 mg vaginal pessary. Complete abortion was also attempted in 5 women who received RU486 together with 2 x 1 mg PG pessaries. Vaginal bleeding followed by complete abortion occurred in 18 of 19 women who received RU486 + 1 mg PG pessary as compared to only 12 of 20 women who received RU486 alone ($P<0.01$). All women who received RU486 + 2 mg Gemeprost had a complete abortion. The onset of crampy abdominal pain (median: 3 vs 4 days) and vaginal bleeding (3 vs 3 days) was similar in the RU486 and RU486 + PG groups, respectively. Slightly less than half the patients in both groups had nausea and/or vomiting, but the incidence did not differ from that occurring prior to treatment. The mean duration (range) of vaginal bleeding [RU486 alone: 10 (0,29) days and RU486 + PG: (5,34) days],and the measured blood loss [RU486: 53 (2,227) ml and RU486 + PG: 81 (32,222) ml] did not differ significantly between the two treatments. It is concluded that the combination of RU486 and a single PG vaginal pessary is a highly effective means of inducing therapeutic abortion in early pregnancy and offers an alternative to surgery.

*Present address:
Department of Obstetrics and Gynaecology
Monash University
Queen Victoria Medical Centre
172 Lonsdale Street
Melbourne, Australia, 3000

Submitted for publication October 23, 1986
Accepted for publication October 28, 1986

Introduction

Therapeutic abortion can be performed effectively and safely by vacuum aspiration of the uterus under local or general anaesthesia up to 12 weeks amenorrhoea (1). However, because complications are related to gestational age and anaesthesia, attempts have been made to develop medical means of terminating pregnancy in the first 8 weeks avoiding the necessity of surgery. Oxytocic agents such as prostaglandins E and F applied locally into the uterus will induce abortion in the first trimester (2,3). A variety of more stable and uterine-specific analogues has been developed which induces abortion in over 90% of women when given by vaginal pessary or intramuscular injection (4-7). However, their widespread acceptance is limited by a relatively high incidence of gastrointestinal side effects.

The myometrium is maintained in a quiescent state throughout pregnancy due to the high levels of progesterone (8). Although antagonism of the biological effect of progesterone by the progesterone receptor antagonist RU486* results in an increase in myometrial activity and uterine bleeding, the incidence of incomplete abortion (about 30%) is unacceptably high (9,10). Recently it has been reported that a higher rate of complete abortion can be achieved in early pregnancy if RU486 is combined with a sub-therapeutic dose of sulprostone - a potent analogue of prostaglandin E_2 (11). In the present study we compare the results of the administration of RU486 alone or in combination with a single vaginal prostaglandin pessary (Gemeprost: 16,16-dimethyl-trans-Δ_2-prostaglandin E_1)** in women in early pregnancy (<56 days amenorrhoea). In this way it was hoped that a highly effective medical means of inducing abortion could be developed with minimal side effects.

Patients and Methods

Forty-five women with pregnancies of less than 56 days amenorrhoea were recruited for the study. Gestational age was assessed by menstrual history and clinical examination, supported by pelvic ultrasound. In all cases, pregnancy was confirmed with an immunological pregnancy test (12).

Patients with evidence of multiple pregnancy or spontaneous abortion were excluded from the study, as were women with medical complications such as cardiovascular or pulmonary disease, allergy or epilepsy. Pregnancy

---

* 17 β-hydroxy-11 β-(4-dimethylaminophenyl)-17α-(1-propynl)-estra-4,9-dien-3-one; Roussel Laboratories, Ltd., Broadwater Park, North Orbital Road, Uxbridge, Middlesex, U.K. UB9 5HP.

** Cervagem; May & Baker, Ltd., Dagenham, Essex, U.K. RM10 7XS.

termination was indicated under the 1967 Abortion Act; local ethical committee approval was granted for the project, and written informed consent was obtained from each woman prior to proceeding with treatment.

Patients were randomly allocated to the study groups. Twenty women rceived RU486 alone, given orally at a dose of 150 mg daily for 4 days. The remainder were also treated with the same dose of RU486 for 4 days, but in addition they received 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester in the form of vaginal pessaries (Gemeprost) 48 hours after commencing the antigestogen. In this group, the first 5 women were treated with 2 x 1 mg of PG, therefore the PG dose was reduced to 1 mg in total.

Prophylactic medication was not prescribed; however, analgesics (paracetamol 1 gm; dihydrocodeine 30 mg; pethidine 100 mg or diamorphine 5 mg), antiemetics (cyclizine 50 mg) or other preparations, such as anti-diarrhoeal agents, were administered as required. No dietary restriction was enforced.

After treatment, arrangements were made for follow-up at 1 week, 2 weeks, 4 weeks and then every 2 weeks until the onset of the next period. Abortion was considered successful at the 2-week visit if there was minimal vaginal bleeding, the cervix was closed, the uterus was normal in size and the serum concentration of human chorionic gonadotrophin had fallen. In the event of treatment failure, the uterus was evacuated under general anaesthesia.

In 24 women (11 on RU486 alone and 13 on RU486 + PG), blood loss was measured using a modification of the alkaline haematin method (13). Soiled pads and tampons were placed in molar sodium hydroxide and thoroughly mixed. Twenty-four hours later, an aliquot was taken and, after filtration, its optical density was measured at 450 nm. Blood loss was then calculated by comparing this with the optical density of a peripheral blood sample similarly processed.

Statistical Analysis

The Wilcoxon rank sum test and the Fisher exact probability test have been used.

Results

All five women receiving the higher dose of PG aborted completely but had crampy abdominal pain. Accordingly, in the remaining women the dose of PG was reduced to 1 mg and the presented data, therefore, refer to those patients who received either RU486 alone (n=20) or RU486 + PG (n=20). In the latter group one woman, in whom treatment was discontinued after 36 hours because of side effects prior to

receiving any PG, has been excluded from analysis. There were no significant differences between the characteristics of the 2 groups (Table).

Table: Patient Characteristics

	RU486 (20)	RU486 + PG (19)
AGE (years)	25 (17,41)	23 (17,38)
HEIGHT (cm)	168 (142,175)	165 (157,173)
WEIGHT (kg)	64 (47,88)	61 (51,68)
PAROUS (%)	5 (20%)	2 (11%)
GESTATION (days)	47 (39,56)	47 (35,56)

Apart from parity, which represents the number of parous women with the percentage in brackets, the data presented show the median and range.

Bleeding followed by complete abortion occurred in only 12 (60%) women in the RU486 group but in 18 (95%) of those women treated in combination with PG ($p<0.01$). At uterine evacuation there was evidence of fetal tissue in all 8 treatment failures in the former group, and in 5 cases the fetal heart was present on ultrasound scanning prior to surgery. The one "failure" in the RU486 + PG group underwent curettage 6 weeks after initial treatment because of persistent mild discharge and an ultrasound appearance consistent with the appearance of minimal debris within the uterus. Necrotic trophoblastic tissue was demonstrated histologically.

There were no differences between the two groups in terms of the onset of crampy abdominal pain or vaginal bleeding. The median (range) day of onset was 2 (0,4) and 3 (0,4) for pain, and 3 (1,6) and 3 (1,4) for bleeding in the RU486 alone and RU486 + PG groups, respectively. In addition, there was no statistically significant difference in the use of analgesics between the treatment groups, with 5 (25%) women in the RU486 group and 9 (47%) in the RU486 + PG group requesting pain relief (N.S.; P=0.09). Only 3 (16%) women, all of whom were in the PG-treated group, required pethidine or diamorphine (N.S.; P=0.1). No serious complications were seen. Eleven (55%) women in the RU486 group and 7 (37%) in the RU486 + PG group complained of nausea during the first 2 days of treatment, whereas in 2 (11%) cases in the latter group this was only noticed after the PG administration. It should be noted, however, that

the incidence of nausea in the 2 groups on the day prior to treatment with RU486 was 58% and 69%, respectively, in the 13 women whose data were available for analysis.

It was possible to record the incidence of vomiting and diarrhoea in the 48-hr period prior to treatment in 13 and 9 women in the RU486 and RU486 + PG groups. Vomiting occurred in 1 of 13 women prior to treatment with RU486 alone and in 3 (33%) of 9 before they received the combined therapy. Duing therapy the number of women with vomiting was 3 (15%) and 6 (32%) in the RU486 alone and RU486 + PG groups, respectively. There was no significant difference between the 2 groups, nor was there any difference before and after treatment in either group, or in the incidence of vomiting before and after the administration of PG in the combined treatment group. Diarrhoea occurred on day 2 in 1 woman in the RU486 alone group, and in 1 woman on day 3 in the RU486 + PG group.

Data were available on blood loss following treatment in 24 women who collected their soiled sanitary protection. The median (range) loss was 53 (2,227) ml (n=11) for the RU486 alone group and 81 (32,222) ml (n=13) for the combined RU486 + PG group (N.S.; $P>0.05$). Five of the 11 patients in the RU486 alone group had undergone uterine evacuation for failure to induce abortion; the median blood loss of the remaining 6 women was 62 (45,227) ml.

One patient in the RU486 alone group had uterine evacuation performed as an emergency because of heavy bleeding 3 days after the cessation of treatment. Although the degree of blood loss was not measured objectively, she was not clinically in shock and it was not considered necessary to give her a blood transfusion. The patient's haemoglobin concentrations before treatment and the day after operation were 12.2 and 11.4 g/dl. Blood transfusion (2 units) was required for another woman treated with RU486 alone who bled heavily following uterine evacuation for treatment failure. No such cases occurred in the RU486 + PG group.

Excluding those women who required operative intervention, bleeding persisted for 10 (0,29) days in the RU486 alone group and 11 (5,34) days in the PG-treated women.

Finally, the interval to the subsequent menstrual period was 39 (23,64) days and 35 (18,48) days in the RU486 and RU486 + PG groups, respectively.

Discussion

While the prostaglandins have been the only compounds investigated on a large scale for the medical interruption

of early pregnancy, the development of the antiprogesterone agents has offered an alternative approach (14). Initial studies have demonstrated that, although the progesterone receptor antagonist RU486 is an effective abortifacient, the incidence of incomplete or unsuccessful abortion when the drug is administered alone is unacceptably high (9,10).

The present study has compared the use of RU486 alone and in combination with 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester, a PG which has been widely used in pessary form to terminate pregnancy in both the first and second trimesters (15-18). Such a combined approach has been advocated on theoretical grounds in that, although the antigestogen may be highly effective at intercepting the pregnancy itself, it may not induce adequate uterine activity to expel the products of conception (19). Furthermore, the fact that antigestogens sensitise the uterus to oxytocic agents (11) should lead to a reduction in the dose of PG required to induce abortion, with a consequent fall in the incidence of unwanted side effects. Using various dose regimens of RU486 in combination with a single low dose of 16-phenoxy-tetranor-PGE_2 methyl sulfonylamide, a complete abortion rate of nearly 100% has been reported (20).

This study has confirmed the success of combined therapy using a fixed dose of RU486 and administering 1 mg of synthetic PG in pessary form after 48 hours. A similar success rate for complete abortion would be anticipated with this PG alone, but at a dosage of 5 x 1 mg pessaries over 12 hours (15,16). Although the administration of PG in this form may be more acceptable to the patient than intramuscular injection, the latter route may be preferred as vaginal drug absorption can be altered in the presence of bleeding. However, the efficacy of treatment was not impaired in the present study in which 10 (53%) women had reported the onset of bleeding prior to pessary insertion.

Most patients experienced some crampy pain during treatment, but there was no difference between the two groups in their requirements for analgesics. We have previously reported a similar requirement using the 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester pessaries alone (17); but in a further group of 30 women undergoing menstrual induction using the same PG, more patients requested some form of analgesic: 26 (87%) versus 9 (47%) for the RU486 + PG group in the present study ($P<0.01$; I.T. Cameron and D.T. Baird, unpublished data). Care must be taken in comparing these results due to the varying subjective awareness of pain in different populations, varying environmental factors, and also to differences in the perception of analgesic requirements on the part of medical and nursing staff.

No serious complications were encountered during therapy, although women in both groups suffered gastrointestinally-associated side effects. Although some patients failed to provide adequate pre-treatment data, it is noteworthy that the incidence of nausea and vomiting did not alter after the commencement of treatment with RU486, nor was it significantly affected by the subsequent administration of PG. Nausea and vomiting are commonly seen in early pregnancy, but diarrhoea is a more specific PG-associated problem; this was only seen in 1 case in each treatment group.

Administration of RU486 was discontinued in 1 woman after 36 hours because of severe nausea and vomiting which pre-dated the start of treatment. She subsequently underwent vacuum aspiration under general anaesthesia without complication, but this does emphasise the potential problem of oral drug administration to some women in early pregnancy.

It has been suggested that in addition to increasing the complete abortion rate, the effect of RU486 plus PG on uterine contractility may also reduce the degree of blood loss (21). In the present study there was no significant difference in the measured blood loss, and the quantity was similar to that previously reported following early abortion with either PG alone or vacuum aspiration (16) and to the expected menstrual loss during a heavy period (22).

One patient in the RU486 alone group suffered acute bleeding necessitating uterine evaucation 12 hours later. Although the bleeding settled with bedrest and a blood transfusion was not required, such a conservative approach would not have been clinically acceptable. Although previous work has outlined the potential danger of excessive bleeding after RU486 treatment (12), blood loss has not been objectively documented. Subjective data on the degree of blood loss between various studies must be interpreted with caution in the light of the finding that up to 50% of women with a subjective complaint of menorrhagia will have a monthly menstrual loss within normal limits (23). The duration of blood loss was again similar for the two treatment groups and did not differ from that seen previously with either PG- or surgically-induced early abortion (16).

In conclusion, the antigestogen RU486 provides a safe method for inducing abortion in early pregnancy with a low incidence of side effects. When used alone there is an unacceptably high incomplete or failed abortion rate; however, the efficacy of treatment approaches that of standard vacuum aspiration when the drug is administered in conjunction with a low dose of PG. Although further studies are required to assess the most appropriate dosage regimens,

such a combined approach to menstrual induction should offer an acceptable alternative to surgery for the interruption of early pregnancy.

Acknowledgements

We are grateful to Sister D. Will, J. McKinnon and staff for help with patient care, and to Miss I. Dunn and Miss M. Seenan for assistance with laboratory assays and typing, respectively. Dr. D. Healy is gratefully acknowledged for his contribution in designing the study. RU486 was kindly supplied by Roussel Laboratories, Ltd.

References

1. Paintin, D.B.: Legal abortion in England and Wales. In: Abortion: Medical Progress and Social Implications. Ciba Foundation Symposium 115, Pitman, London, 1985, pp 4-12.

2. Mocsary, P. and Csapo, A.I.: Menstrual induction with $PGF_{2\alpha}$ and PGE_2. Prostaglandins 10:545-547, 1975.

3. Mackenzie, I.Z., Davies, A.J., Embrey, M.P. and Guillebaud, J.: Very early abortion by prostaglandins. Lancet i:1223-1226, 1978.

4. Bygdeman, M., Bremme, K., Christensen, N., Lindstrom, V. and Green K.: A comparison of two stable prostaglandin E analogues for termination of early pregnancy and for cervical dilatation. Contraception 22:471-483, 1980.

5. Csapo, A.I., Peskin, E.G., Pulkkinen, M., Laajoki, V., Kivikoski, A., Lampe, L., Godeny, S., Szeverenyi, M., Herczeg, J., Resche, B. and Bacos, L.: "Menstrual induction" with sulprostone. Prostaglandins 24:657-665, 1982.

6. Bygdeman, M. and Green, K.: Menstrual regulation: Medical techniques. In: Pregnancy Termination - Procedures, Safety and New Developments (G.I. Zatuchni, J.J. Sciarra and J.J. Speidel, Eds.). Harper & Row, Philadelphia, 1978, pp 69-77.

7. Bydgeman, M., Green, K., Lundstrom, V., Ramadan, M., Fokiou, S. and Bergstrom, S.: Induction of abortion by vaginal administration of 15(S)15-methyl prostaglandin $F_{2\alpha}$ methyl ester. A comparison of two delivery systems. Prostaglandins 12:Suppl. 27-51, 1976.

8. Csapo, A.I., Pulkkinen, M.O. and Kaihola, H.L.: The effect of luteectomy-induced progesterone-withdrawal on the oxytocin and prostaglandin response of the first

trimester pregnant human uterus. Prostaglandins 4:421-429, 1973.

9. Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E. and Baulieu, E.E.: The effects of an antiprogesterone steroid in women: interruption of the menstrual cycle and of early pregnancy. Comptes Rendus 294: 933-940, 1982.

10. Kovacs, L., Sas, M., Resch, B.A., Ugocsai, G., Swahn, M.L., Bygdeman, M. and Rowe, P.J.: Termination of very early pregnancy by RU486 - an antiprogestational compound. Contraception 29:399-410, 1984.

11. Swahn, M.L., Cekan, S., Wang, G., Lundstrom, V. and Bygdeman, M.: Pharmacokinetic and clinical studies of RU486 and human fertility regulation. In: The Antiprogestin Steroid RU486 and Human Fertility Control (E.E. Baulieu and S.J. Segal, Eds.). Plenum Press, New York, 1985, pp 249-258.

12. Hobson, B.M.: Pregnancy diagnosis using the pregnosticon haemagglutination inhibition test. Brit. J. Obstet. Gynaecol. 75:718-723, 1986.

13. Hallberg, L. and Nilsson, L.: Determination of menstrual blood loss. Scand. J. Clin. Lab. Invest. 16:244-248, 1964.

14. Healy, D.L. and Fraser, H.M.: The antiprogesterones are coming: menses induction, abortion, and labour? Brit. Med. J. 290:580-581, 1985.

15. Takagi, S., Sakata, H., Yoshida, T., Den, K., Fujii, T.K., Amemiya, H. and Tomita, M.: Termination of early pregnancy by ONO-802 suppositories (16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester). Prostaglandins 15:913-919, 1978.

16. Smith, S.K. and Baird, D.T.: The use of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester (ONO 802) vaginal suppositories for the termination of early pregnancy. A comparative study. Brit. J. Obstet. Gynaecol. 87:712-717, 1980.

17. Cameron, I.T., and Baird, D.T.: The use of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester (Gemeprost) vaginal pessaries for the termination of pregnancy in the early second trimester. A comparison with extra-amniotic prostaglandin E_1. Brit. J. Obstet. Gynael. 91:1136-1140, 1984.

18. Cameron, I.T. and Baird, D.T.: A controlled release form of 16,16-dimethyl-trans-Δ_2-PGE_1 methyl ester for early abortion. Contraception 33:121-125, 1986.

19. Baulieu, E.E.: RU486: an antiprogestin steroid with contragestive activity in women. In: The Antiprogestin Steroid RU486 and Human Fertility Control (E.E. Baulie and S.J. Segal, Eds.). Plenum Press, New York, 1985, pp 1-25.

20. Swahn, M.L., Cekan, S., Wang, B., Lundstrom, V. and Bygdeman, M.: Clinical experience with antiprogestins. ICMR/WHO Scientific meeting; Bangalore, India, 1985.

21. Baulieu, E.E.: Contragestion by antiprogestin: a new approach to human fertility control. In: Abortion: Medical Progress and Social Implications. Ciba Foundation Symposium 115, Pitman, London, 1985, pp 192-210.

22. Hallberg, L., Hogdahl, A.M., Nilsson, L. and Rybo, G.: Menstrual blood loss - a population study. Acta Obstet. Gynael. Scand. 45:320-351, 1966.

23. Fraser, I.S., Pearse, C., Shearman, R.P., Elliott, P.M., McIveen, J. and Markham, R.: Efficacy of mefenamic acid in patients with a complaint of menorrhagia. Obstet. Gynaecol. 58:543-551, 1981.